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BEARING ON HEREDITY AND VARIATION

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CHROMOSOME ABNORMALITIES IN AN INDIVIDUAL OF CHORTHIPPUS LONGICORNIS (ACRIDIDAE)

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Received June 10, 1947

CHROMOSOME abnormalities in the Acrididae so far described have had to do mainly with the so-called fusion of rod-shaped chromosomes to form V-shaped ones, with an accompanying reduction of chromosome number. The 17-chromosome forms of the Truxalinae; Chorthippus, Chloealtis, Chrysochraon, and the 19-chromosome form Neopodismopsis (*Rothfels*, unpublished) have, no doubt, been derived from twenty-three-chromosome forms in this way. They illustrate the so-called "Robertson Law." In all probability, these V-chromosomes originated not by simple fusions but by very unequal reciprocal translocations leading to the elimination of short segments from both rod chromosomes concerned and including only a single centromere from one of the two fusing chromosomes. The minute chromosome formed as the second product of the reciprocal translocation must then have been eliminated. The multiple chromosomes of Hesperotettix and Mermiria described by McCLUNG (1927) also appear to have been derived in this way (see MULLER 1940, p. 223).

Another type of chromosome modification which appears to be common in western species of Circotettix and Trimerotropis (CAROTHERS 1931, KING 1923, HELWIG 1933) is a shift in the centromere from near the end to a submedian position. This gives rise to metacentric chromosomes without any reduction in number or increase in size. In these genera, as in Hesperotettix and Mermiria, the modification has not become fixed and varies from individual to individual.

The change in position of the centromere in Circotettix and Trimerotropis has been ascribed to (a) a pericentric inversion, or (b) a lateral shift in the centromere (HELWIG, 1933, WHITE 1940). As pointed out by WHITE, the former alternative appears to be the more probable but, as far as I am aware, no direct observational evidence has been advanced in support of either.

Evidence of paracentric inversions in the Orthoptera in the form of bridges, is not abundant in the literature, but the phenomenon is a common one and such inversions must be practically universal in grasshopper species. Heteromorphic bivalents due to unequal lengths have also been found in a number of forms (see WHITE 1945, p. 108) and must, I believe, be almost equally widespread.

When we look for evidence in the Acrididae for another common source of abnormality, viz. reciprocal translocations, we find it confined to reports by CAROTHERS (1931), WHITE (1940) and HELWIG (1942). In these cases the evidence was confined to first meiotic metaphase and anaphase configurations. On the basis of its repeated occurrence in more than one year in the same

locality, WHITE considered the cases reported by him as systemic in nature, but the grounds for this view appear to be insufficient. The percentage of multivalents (quadrivalents) found by him in any one individual was low, and investigations in this laboratory indicate that recurrent non-systemic translocations in grasshoppers are by no means so rare as has been assumed.

MATERIAL AND METHODS

The material here reported upon is from a male of *Chorthippus longicornis* collected, along with many others, in the neighborhood of Port Carling, Muskoka, Canada, in August 1945. All material collected at that time was being used in preliminary tests on the effects of dilute aqueous solutions of colchicine on mitosis and meiosis, the solutions being injected into the abdomens of the grasshoppers, and the testes fixed and stained at intervals up to five days after the treatment. It is not proposed to report on the effects of the colchicine treatment at this time, except in so far as it affected the material from this one individual. As in all the plant material so far reported upon, the treatment led to complete inhibition of spindle formation, so that if the nuclei were not already in anaphase no chromosome separation took place. The result was a huge accumulation of spermatogonial and first meiotic metaphases. Meiotic prophases, more especially early and late diplophases, also showed up very clearly. Mitotic and meiotic anaphases were almost absent and owing to the marked shortening and thickening of the chromosomes were of no use for study. The shortening and thickening of the chromosomes at metaphase and the failure of the V-shaped chromosomes to bend at the centromere is a further characteristic result of treatment with colchicine, as can be seen in Plate 2, figure 2 and Plate 4, figures 11 and 12.

The testis was dissected out into Ringer's solution, the fatty tissue surrounding it removed and the whole testis fixed in modified Levitsky's chromformol (equal parts of one percent chromic acid and ten percent formalin). It was then stained *in toto* by the Feulgen method, individual tubules were

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PLATE 1

Explanation of Figures

FIGURE 1.—Pachyphase configuration showing pairing relationships of the four chromosomes involved in the multivalent, its individual elements being labeled.

FIGURE 2.—Early diplophase configuration showing, below, the result of a crossover in the region of the pericentric inversion and, above, association of the chromosome $1^{2,7}$ with a 7^{1} chromosome.

FIGURE 3.—Early diplophase of same stage as figure 2. The homologous ends of the two arms below have formed a subterminal chiasma which, on terminalization, will form a ring such as shown at the bottom of multivalents reproduced in figures 4, 6 and 7.

FIGURE 4.—Late diplophase. Complete complement showing a univalent above and to right.

FIGURE 5.—Late diplophase. A complement showing univalent above and to right. One of the small bivalents appears to have been attached to or hidden by the X chromosome below to left.

FIGURES 6, 7, 8.—Late diplophase—different forms of the multivalent. In figures 6 and 8 chromosome 7^{1} is included; in figure 7 it is not.

Magnification in all cases approximately 1600 diameters.

PLATE I

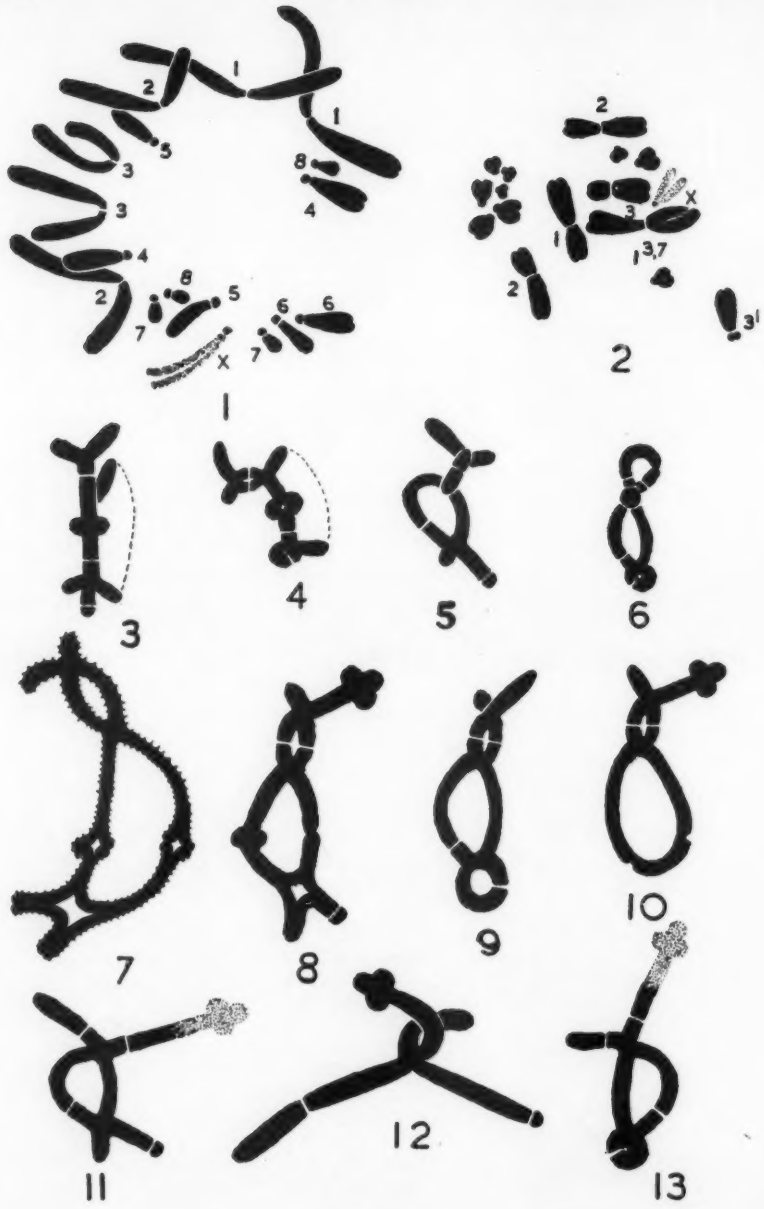

PLATE 2

squashed on slides in 50 percent acetic acid, and the slides were transferred through dioxane to dioxane balsam.

OBSERVATIONS

Before proceeding to a consideration of the abnormalities observed, it is necessary to refer briefly to the normal chromosomes, although their morphology has been described by previous authors. An examination of Plate 2, figure 1 and Plate 4, figure 10, reproducing respectively an early and a late spermatogonial metaphase, shows the presence of three metacentric chromosome pairs which can be readily distinguished from one another and which are numbered 1–3 in descending order of length. Following these in descending order are five pairs of acrocentric chromosome pairs (4–8 inclusive) plus, of course, a single X chromosome.

A study of spermatogonial metaphases in the individual under examination revealed a marked change in two of the metacentric chromosomes, one belonging to pair 1 and the other to pair 3. The longer of these, labeled $1^{3.7}$ in Plate 2, figure 2, and Plate 4, figures 11 and 12, is longer than the normal chromosome 1 and has its centromere approximating more closely to the median position. This means that, although both arms have been lengthened, the shorter one has been elongated more than the longer one.

The altered chromosome 3, labeled in the same figures as 3^1, is somewhat shorter than its partner and has its centromere in an acrocentric instead of the

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PLATE 2

Explanation of Figures

FIGURE 1.—A normal early spermatogonial metaphase showing the different types of chromosomes.

FIGURE 2.—Spermatogonial metaphase in the abnormal individual with only the long pairs labeled. Irregular arrangement, shortening of chromosomes and failure to form V-shape is due to colchicine treatment.

FIGURES 3 and 4.—First meiotic metaphase multivalents showing failure of chiasma formation between chromosome 3^1 and chromosome 1.

FIGURE 5.—Multivalent at first metaphase showing result of chiasma formation at one side of the inversion loop. Chromosome 7^1 not involved here.

FIGURE 6.—Multivalent at first metaphase showing terminal rings due to formation of subterminal chiasmata between (a) 7^1 and 7 and (b) the two homologous ends due to chiasma in inversion.

FIGURE 7.—Late diplophase multivalent showing incomplete chromatid below and unterminalized chiasmata at sides.

FIGURE 8.—First metaphase multivalent showing equal open arms below and chiasma between $1^{3.7}$ and 7^1 above.

FIGURE 9.—First metaphase multivalent with ring below and no association of 7^1 above.

FIGURE 10.—First metaphase multivalent showing result of failure of chiasma formation in the inversion but retention of terminal chiasma distal to inversion.

FIGURES 11 and 13.—First metaphase multivalents showing negative heteropycnosis in the region of the $1^{3.7}$ and 7^1 association.

FIGURE 12.—First metaphase multivalent in which chiasmata in the chromosome 3 part of the configuration are entirely lacking.

Magnification in all cases approximately 1600 diameters.

usual metacentric position. At first sight one might be tempted to ascribe this apparent change of position of the centromere to a deletion in the shorter arm. However, the long arm of this chromosome is distinctly longer than the long arm of its normal partner, and pairing relations and diplophase configurations, to be described, show clearly that the centromere shift is due to a pericentric inversion asymmetric with reference to the centromere.

The pairing relationships of these translocated chromosomes $1^{3,7}$ and 3^{1} with their normal partners are shown clearly in Plate 1, figure 1 and Plate 3, figure 1, both representing the same structure. In the lower right corner of each figure is shown the typical inversion loop with the approximate position of the two centromeres of the No. 3 chromosomes, marked at c in Plate 1, figure 1. This position has been obtained by using the relative lengths of the two arms of 3 and 3^{1} respectively at premeiotic metaphase. Gene loci are indicated in the normal third chromosome by the letters A–I, and corresponding gene loci of translocated chromosome 3^{1} are marked in the region where the two synapse. The univalents are already split at this stage but they are shown single in Plate 1, figure 1, to simplify the drawing.

Plate 1, figure 2, and Plate 3, figure 3 both show the same early diplophase configuration where there has been a crossing over in the region of the inversion between gene loci G and H. As a result there has been formed, as one of the products, an incomplete chromatid in which the gene locus I has been duplicated, an inevitable result of a cross-over in this type of inversion. A comparison of Plate 1, figure 2 with figure 1 of the same plate will show that the position given to the gene loci in this region is approximately correct, although the loci seem too far apart relative to their distance apart in the region A–G. This must be due to a greater stretching of the chromonemata in the region G–I owing to the rather heavy pressure applied in squashing. Farther up can be seen two further chiasmata, one between chromosome $1^{3,7}$ and chromosome 3, the other between chromosome 1 and chromosome 3^{1}. The latter is already almost terminalized.

The presence of similar ends in the deficient chromatid leads to the possibility of chiasma formation in the region of the I locus as is shown in Plate 1, figure 3 and Plate 3, figure 4. Terminalization of this chiasma gives the terminal ring shown in Plate 3, figures 5 and 7 and in many of the later diplophases (Plate 2, figures 4, 6 and 9, and Plate 3, figures 8 and 9, Plate 4, figure 4). Metaphase configurations showing the same ring are reproduced in Plate 1, figures 4, 6, 8 Plate 2, figure 13, and Plate 4, figure 7. The open arm structure which results from a failure of chiasma formation in this region is shown for early prophase in Plate 1, figure 2 and Plate 3, figures 2 and 3, for later prophase in Plate 1, figure 7, Plate 2, figures 5, 8 and 11 and Plate 4, figures 5, 6 and 8. The relative lengths of the two arms may vary greatly depending upon just where, in the inversion, the chiasma has been formed. A chiasma far from the middle of the inversion loop is responsible for the extreme asymmetry shown in Plate 2, figure 5 and Plate 3, figure 3. It is also evident in such configurations as are shown in Plate 1, figures 4 and 8, and Plate 2, figure 6, where the position of the centromere shows the asymmetry.

Where chiasma formation fails in the inversion but is present in the region distal to it, one obtains, on terminalization, a large ring at the bottom of the number 3 chromosome end of the configuration. Illustrations of this are given in Plate 2, figure 10 and Plate 4, figure 9. Where chiasmata fail entirely in this part of the complex, a figure such as that shown in Plate 2, figure 12 results. These two last types are comparatively rare, the former having been found in about ten percent, the latter in about one percent of the configurations examined. Either one of these configurations would produce viable gametes in 50 percent of the cases as far as the genes in chromosomes 1 and 3 are concerned.

As will be noted on an examination of first metaphase figures in the plates, the position of the centromere is strikingly evident in the case of all of the four long chromosomes involved, as a more or less complete unstained transverse band on the chromosomes. This enables one to identify the different members of the configuration with great exactitude and to establish the fact that the relationship to each other in the multivalent remains constant. It also enables one to fix approximately the position of terminalized chiasmata where, as at first metaphase, they are usually not visible.

When we come to chiasma formation between the number 3 and the number 1 chromosomes, at least one is always present, thus ensuring the formation of a quadrivalent. Usually chiasmata are formed on both sides, that is between chromosome $1^{3,7}$ and chromosome 3 on the one side and chromosome 1 and chromosome 3^{1} on the other. These lateral chiasmata are shown in most of the first meiotic prophase figures as for example Plate 3, figures 2–8. In some cases the chiasma on one side becomes terminalized at an early stage of diplophase as in figure 2, in others both persist to a comparatively late stage as in figure 6. At times terminalization is incomplete at first metaphase as in Plate 2, figure 8 to the left and Plate 3, figure 8 to the left.

Where the chiasma fails on one side we obtain configurations like those illustrated in Plate 2, figures 3 and 4, the arms in which chiasma formation has failed here being joined by dotted lines. The exact position of terminalized chiasmata can commonly not be identified at metaphase but occasionally it can be made out as in Plate 2, figure 8. Here the chiasma between chromosome $1^{3,7}$ and chromosome 3 has remained unterminalized.

Turning to the part of the configuration involving a translocation between a chromosome 1 and one of the short acrocentric chromosomes, measurements indicate with a high degree of probability that this latter is a number 7 chromosome. It is also apparent that the number 1 chromosome which is involved in this translocation has also taken part in the 1–3 translocation. The drawing, from which Plate 1 figure 1 has been reduced, showed a difference in length, taken from a point in close synapsis, of 2.5 cm between this chromosome and the other number 1 chromosome. This is about the same length as the normal chromosome number 7 at the same magnification and approximately the same stage of pachyphase. The accompanying diagram gives the approximate relative lengths of the translocated pieces and of the resultant translocated chromosomes.

It has not been possible to identify any chromosome corresponding to the

small 7^1 chromosome represented in the diagram, which must have been one of the products of an unequal reciprocal translocation. As will be seen from an examination of Plate 4, figure 10, in a spermatogonial metaphase of a normal individual, the 17 chromosomes characteristic of the species can be made out

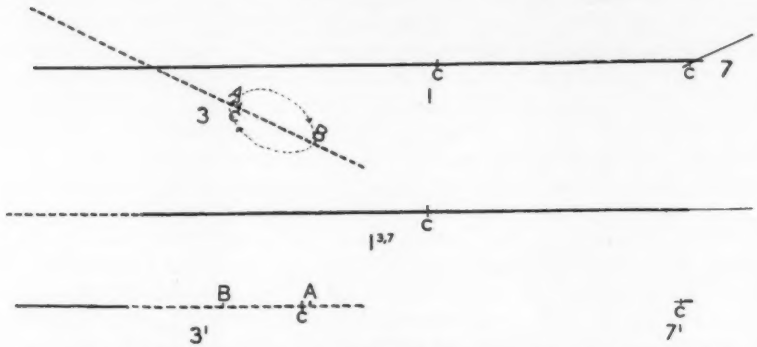

Diagram showing the main translocations between chromosomes 1, 3, and 7 with products. The centromere is marked by C, the inverted section in chromosome 3 by AB.

without any difficulty. On the other hand, figures 11 and 12 on the same plate, which show similar metaphases from the abnormal individual, show only 16 chromosomes or 15 plus X. One of the short chromosomes must be present in the haploid number. Also if we examine complete first meiotic division nuclei, we find that first meiotic prophases and metaphases also appear to contain a univalent (upper right in Plate 1, figures 4 and 5) in those cases where there is no evidence of synapsis of the short chromosome with the end of chromosome $1^{3,7}$. In case of synapsis there is one less unit than in cases where no synapsis occurs. The total number of units is seven instead of the eight that are other-

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PLATE 3

Explanation of Figures

FIGURE 1.—Compare with Plate 1, figure 1.

FIGURE 2.—Early prophase showing a complete complement. One of the short bivalents looks as if attached to an arm of the multivalent at extreme top but really lies beneath it.

FIGURE 3.—Compare with Plate 1, figure 2.

FIGURE 4.—Compare with Plate 1, figure 3.

FIGURES 5 and 7. Early prophase multivalents showing complete rings below owing to terminalization of subterminal chiasma.

FIGURE 6.—Late diplophase showing complete complement. The univalent 7^1 is above to left. Compare with Plate 2, figure 7.

FIGURE 8.—Late diplophase showing complete complement. The univalent 7^1 is above to right. Compare with Plate 1, figure 4.

FIGURE 9.—Late diplophase showing complete complement. Univalent 7^1 attached to right of multivalent, thus reducing separate units from 8 to 7. Compare figure 8.

Magnification in all cases approximately 950 diameters.

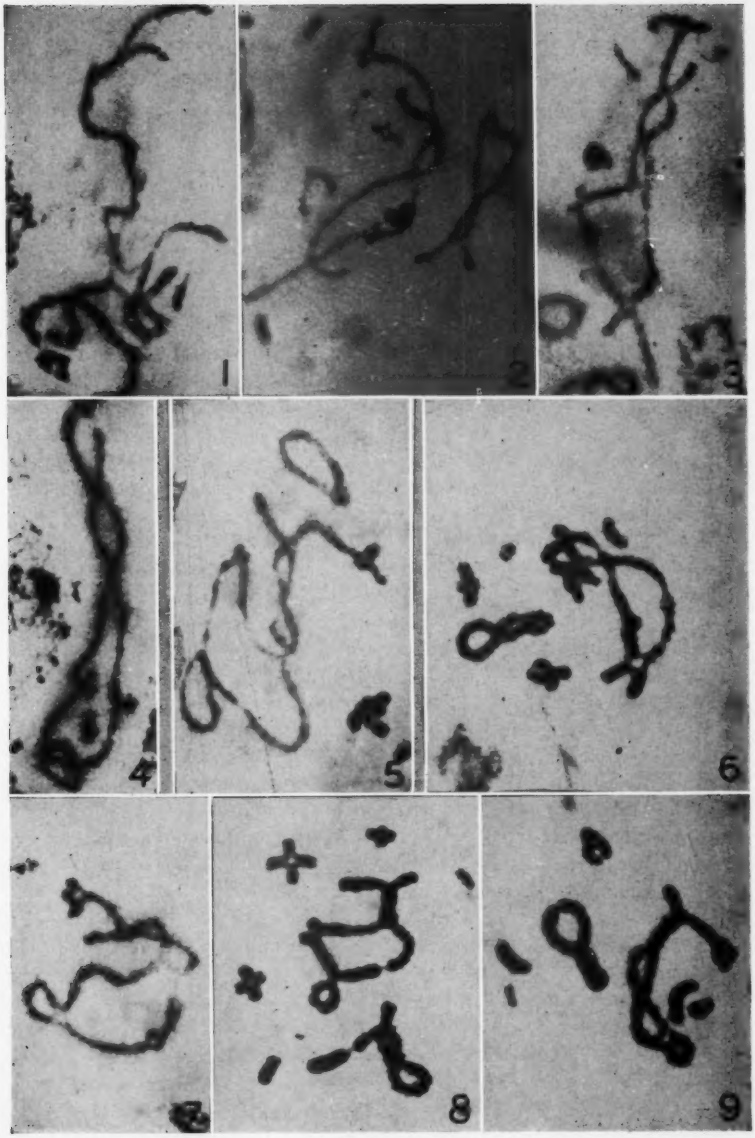

PLATE 3

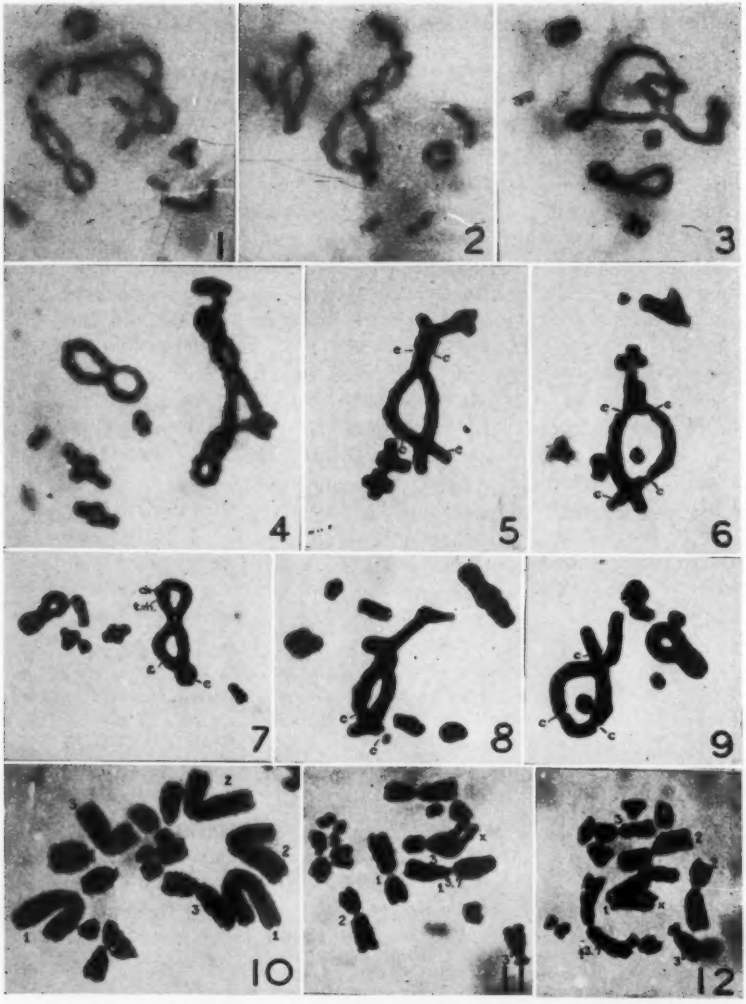

PLATE 4

wise present or the nine that are present at first meiotic metaphase in normal forms.

At first it would appear that the separate short chromosome involved at the top of configurations illustrated in Plate 1, figure 2 and Plate 3, figures 2, 3, 5 and 7 must be a normal chromosome 7. However, when we examine Plate 2, figure 6 and Plate 4, figure 7 we find a terminalized chiasma forming a ring. As pairing and chiasma formation take place only between homologous parts there must be at one end of the small chromosome 7, a small terminal portion of chromosome 1, or at the end of chromosome 1 a small section from the short arm of chromosome 7. It is true, if we postulate the presence of the small translocated chromosome represented in the diagram, its interpolation in the sequence would give us a sexavalent, but, as already stated, there is no evidence of the retention of this small chromosome. Plate 4, figure 7 especially shows the terminal chiasma between the small chromosome 7 and chromosome 1 (t. ch), and close above it the unterminalized chiasma (ch) between the chromosome 7 and chromosome $1^{3,7}$. We are, therefore, forced to conclude that this configuration is a quinquevalent not a sexavalent.

It seems to me probable that a chromosome 7 carries this last translocation 1. The portion of the chromosome lost by such a translocation would have to be in the short arm of chromosome 7 which, as it does not extend much beyond the centromere, is likely to be relatively inert genically. The loss of an end section of a chromosome 1 would be likely to lead to greater genic disturbance and a less viable chromosome. Further, it seems necessary to postulate that this translocation took place in the opposite parent of the heterozygote from the one providing the original translocations between chromosomes 1, 3 and 7. The chromosome 7^1 found in the nuclei of this individual therefore consists of a long arm and centromere derived from chromosome 7 and a short segment from the end of chromosome 1 which replaces an end segment of the short arm of 7. We are, however, not at the end of

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PLATE 4

Explanation of Figures

FIGURES 1–3.—Mid-diplophase nuclei in which the whole complement can be made out with some degree of certainty.

FIGURE 4.—Late diplophase—compare with Plate 1, figure 6.

FIGURE 5.—First metaphase multivalent showing open arms below and attachment of 7^1 above.

FIGURE 6.—First metaphase multivalent showing negative heteropycnosis above.

FIGURE 7.—Complete complement at first metaphase. Multivalent shows ring above and beneath. Compare with Plate 2, figure 6.

FIGURE 8.—Complete complement at first metaphase.

FIGURE 9.—First metaphase multivalent showing failure of chiasma formation within the inversion. Compare Plate 2, figure 10.

FIGURE 10.—Normal late spermatogonial metaphase with metacentric chromosomes numbered (photo K. ROTHFELS). Compare with Plate 2, figure 1.

FIGURES 11 and 12.—Spermatogonial metaphases with normal and translocated long chromosomes numbered. Compare with Plate 2, figure 2.

Magnification in all cases approximately 950 diameters.

the phenomena connected with the 1–7 association. As is evident from Plate 2, figures 11 and 13 and Plate 4, figure 6, the 7-portion of $1^{3,7}$ and the 7^{1} may show negative heteropycnosis. This is not universally the case as can be seen from Plate 2, figures 5, 10 and 12. It was not confined to any particular testicular tubule, so it is hard to see how any difference in environmental conditions or any difference of fixation could account for this phenomenon. For the present I shall have to leave it as an uninterpreted observation. This heteropycnosis is, at least partially, due to a less compact coiling of the chromonemata as is evidenced by the greater length of the end involved in these figures as compared to the length in comparable figures such as Plate 2, figure 8.

DISCUSSION

The various configurations which have been described and illustrated must, in almost every case, yield a high proportion of non-viable gametes. Reciprocal translocation between chromosomes 1 and 3 would, in itself, lead to 50 percent non-viability unless some mechanism is present favoring proper disjunction. Lack of first meiotic anaphase figures prevents any decision on this point. Failure of pairing of chromosome 7^{1} with chromosome $1^{3,7}$, which was apparent in about 50 percent of the 318 nuclei examined for this purpose, would almost certainly lead to failure of proper distribution of the univalent. Finally, crossing over in the inversion region of chromosome 3 would lead to duplications and deficiencies in two of the four chromatids concerned. This was found to take place in 89 percent of the configurations examined for the purpose. The only types that are free from this disability are those in which ends near the inversion are free, as in Plate 2, figure 12 representing one percent of the total or those in which a chiasma is formed only distal to the inversion as in Plate 2, figure 10, and Plate 4, figure 9. These represented about ten percent of the total.

Notwithstanding these facts, a large number of the spermatozoa present in the preparations appeared to be quite normal. There were a greater number of giant spermatozoa than are usually found, but they still formed a small percentage of the total. I need hardly state that the individual itself showed no morphological peculiarity such as would strike one in an ordinary examination. As stated, I was studying not morphological differences but the effect of colchicine and could hardly believe my eyes when the chromosome abnormalities here described met them.

All the abnormalities described above, with the possible exception of the persisting 7^{1} translocation, must have arisen in the parental generation of this complex heterozygote. The various chromosomal changes need not have arisen during one mitotic cycle. They may have been scattered over a series of successive cell divisions. They must, however, almost certainly have arisen during one generation for all the changes with the exception of the final 7^{1} translocation are such as to cause very heavy reduction in fertility. The heterozygote cannot have arisen as a result of a chance union of two distinct cytological races differing in gene arrangement as may happen for example in *Drosophila azteca* (DOBZHANKY and SOCOLOV 1939). It must be emphasized that this was a single individual collected in an area from which large numbers have been examined.

All the other individuals collected and studied cytologically, whether they had been treated with colchicine or not, showed no more striking hybrid character than the presence of paracentric inversions as evidenced by first and second anaphase bridges. This type of abnormality is likely to be found in almost any grasshopper population if one deals with any considerable number of individuals.

The pericentric inversion here described is, as far as I am aware, the first one that has been properly documented by a study of different meiotic stages. The presence of pericentric inversions has, for the most part, been inferred from a shift in the position of the centromere observable at mitotic metaphase as in Ichthyophis (SESHACHAR 1939) or at first meiotic metaphase and anaphase as in Circotettix and Trimerotropis (CAROTHERS 1921, KING 1923, HELWIG 1929). Nothing has been reported on the mode of transmission of the heteromorphic chromosomes in the first case but, in the case of Circotettix and Trimerotropis, the presence of these inversions apparently does not affect fertility. MULLER (1940) has suggested that this is due to a localization of chiasmata in chromosome sections outside the region of inversion and more particularly in subterminal regions. The bases for this conclusion are the facts that the products of disjunction at first meiotic anaphase are normal and that no reduction of viability occurs. It should be emphasized, however, that, in each of the cases cited, the shift of the centromere has been from an acrocentric to a metacentric position and, as the short arm of the acrocentric chromosomes in these forms is relatively very short, there will be a very short terminal region left in which a chiasma outside the inversion could be formed. This will greatly reduce the chances of chiasma formation in this region. At metaphase, in these forms, the bivalents are usually maintained by a terminal chiasma at the end of the long arm farthest removed from the centromere. However, an examination of Carother's figures (1921, text figure A2 and 4) shows that a chiasma may be formed in the short arm of the chromosomes presumed to contain the inversion, while HELWIG (1929, p. 5) states that this happens quite frequently. In his later paper (1942) HELWIG expresses doubt that a change in position is due to a pericentric inversion on the ground that irradiation, which would be expected to produce an inversion effect never does so. As apparently no chiasmata are formed in the short arm when these chromosomes have their normal acrocentric structure, the observations suggest a lateral shift of the centromere rather than an inversion. If we postulate the shift of a small piece of the chromosome immediately surrounding the centromere, this would not seriously affect chiasma formation, but would affect the direction in which some chiasmata would move in terminalization. Such a shift would involve three breaks in the chromosome instead of the two required for an inversion but it has been shown in this paper that three breaks are not an excessive number to expect in grasshopper material. CAROTHERS, HELWIG and KING unfortunately do not give any figures illustrating stages before first meiotic metaphase but the metaphase figures show clearly that chiasmata are by no means localized in the normal chromosomes of species of Circotettix and Trimerotropis in general. It seems clear that the whole

question of centromere shift in grasshoppers, and in other forms where it has been reported, requires reexamination, using a more suitable cytological technique than has heretofore been employed.

Summary

1. An individual of *Chorthippus longicornis* has been found to be heterozygous for no less than four chromosome abnormalities.

2. One of the longest chromosomes (chromosome 1) shows a reciprocal translocation with one of the third longest chromosomes (chromosome 3) at one end and a translocation involving most of one of the second shortest chromosomes (chromosome 7) at the other end. The resulting chromosome $1^{3,7}$ is considerably longer than its partner with its centromere occupying a more nearly median position.

3. The translocated chromosome 3 (3^{1}) contains, in addition, a pericentric inversion leading to a shift of the centromere from a submedian (metacentric) to a subterminal (acrocentric) position. Previous centromere shifts in grasshoppers (Trimerotropis, Circotettix) and amphibia which have been accounted for by the presence of a pericentric inversion have been from a subterminal to a submedian position. Moreover, the presence of a pericentric inversion has been postulated solely on the basis of a change in position of the centromere and the possibility of a lateral shift without any inversion is not excluded In the present case early pachyphase and diplophase configurations show conclusively that the pericentric inversion is involved Whereas, in the grasshoppers Trimerotropis and Circotettix, if the postulated pericentric inversions exist, crossing over apparently does not take place within them, in the Chorthippus individual, crossing over in the inversion has taken place in approximately 90 percent of the configurations examined.

4. The reciprocal translocation which involves chromosome 1 and chromosome 7 has led to the transfer of most of chromosome 7 to the end of chromosome 1 and must have resulted in the formation of a minute chromosome involving the centromeric region of 7 and a very small piece from the end of 1. This minute chromosome appears to have disappeared, probably in one of the two parents from which this individual was derived. There is no evidence of it either in the premeiotic metaphase, in the multivalent or as a univalent during the first meiotic division. However, there is present a translocated 7^{1} chromosome which must have arisen in the opposite parent of the heterozygote, by an exchange of a minute terminal part of the short arm of a chromosome 7 with a short terminal section of chromosome 1. This allows for pairing on the one side with the 7 end of chromosome $1^{3,7}$ and on the other side with the end of untranslocated chromosome 1 to form the distal ring found in about 25 percent of the configurations studied. Where chromosome 7^{1} does not pair on either side, it appears as a univalent. Where it pairs solely with chromosome $1^{3,7}$ it forms a cross at the end of this chromosome. This has been found in about 28 percent of the cases examined. Not infrequently a negative heteropycnosis is found involving the translocated end of chromosome $1^{3,7}$ and chromosome 7^{1} forming the cross. No explanation for this can, at present, be suggested, but it

is at least partly, due to a less contracted coiling of the chromonemata in this region.

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THE INHERITANCE OF AMYLACEOUS SUGARY ENDOSPERM AND ITS DERIVATIVES IN MAIZE

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SOME years ago in connection with a maize improvement program in Texas, several thousand self-pollinations were made in Surcropper, a variety of field corn widely grown in the Southwest. Among the self-pollinated ears were a number which segregated for a type of sugary endosperm which appeared to be, and later proved to be, different from the ordinary sugary endosperm familiar to all maize geneticists. The inheritance of this new type of endosperm, named amylaceous sugary, was briefly recorded in the "Maize News Letter" which served the purpose of bringing the character to the attention of other students of maize genetics. Now, however, that CAMERON (1947) has made a comprehensive study, which is reported separately in this journal, of the chemistry of carbohydrate development in types of endosperm derived from amylaceous sugary and its cross with sugary it is desirable to publish the accumulated data which bear upon the inheritance of amylaceous sugary and its derivatives.

DESCRIPTION OF AMYLACEOUS SUGARY

The new type of sugary endosperm was called amylaceous sugary because it resembles in its general aspects the description and illustration of a variety of sugary maize which STURTEVANT (1887) termed *Zea amyleasaccharata* or starchy-sweet corn. The name itself serves as a brief description since the most conspicuous difference between amylaceous sugary and ordinary sugary lies in the somewhat starchy appearance of the endosperm which in amylaceous sugary, especially in the lower half of the seed, is less translucent and less wrinkled than in sugary. This is illustrated in figure 1 in which the two types isolated in homozygous condition from related crosses are compared.

INHERITANCE OF AMYLACEOUS SUGARY

Two major genes are involved in the inheritance of amylaceous sugary. This is indicated by the original selfed ears of Surcropper some of which segregated in ratios approximating 3:1, others in ratios approximating 15:1. When starchy seeds from the latter were grown, three types of ears, (1) not segregating, (2) segregating approximately 3:1, (3) segregating approximately 15:1, occurred in the ratio expected when genes which act as duplicate factors are involved. Fifteen ears gave exactly the theoretical 7:4:4 ratio with respect to these three genotypes.

When amylaceous sugary was crossed with an unrelated non-sugary stock

* The majority of the data reported in this paper were obtained by the writer while on the staff of the TEXAS AGRICULTURAL EXPERIMENT STATION.

all of the F_1 ears (F_2 endosperm generation) segregated in a ratio of approximately 15:1 (267 sugary seeds in a population of 4539). When F_1 plants were backcrossed by amylaceous sugary, segregation followed a 3:1 ratio (374 sugary seeds in a population of 1478).

It should, perhaps, be stated that these data are based on selected ears in which the distinction between starchy and sugary seeds was clear-cut and in which classification was readily made. In some ears, which were excluded, there was an intergradation of the starchy and sugary seeds to such an extent that a completely accurate classification was impossible. Modifiers affecting the expression of the amylaceous sugary character are apparently involved. In spite of this fact, the segregation is clear-cut in the majority of the ears and the data indicate that two independently inherited genes are involved. One of these is called "dull" (*du*) because it was found that in the homozygous condition, separated from the second gene, it gave, in some progenies, a distinctive dull appearance to the seeds. The second gene is designated as su^{am} because it is, as shown below, allelic to *su*.

Anticipating the data to be presented later, it can be said that amylaceous sugary has the genetic composition su^{am} su^{am} *du du*. Ordinary sugary is *su su Du Du* while the normal starchy condition is *Su Su Du Du*. The effects of either recessive acting alone are not usually visibly discernible although segregation for the dull gene is apparent in some progenies. The combined effect of the two recessive genes, however, produces a type of sugary endosperm which on the one hand is readily distinguishable from starchy *Su Su Du Du* and on the other hand, is clearly different from ordinary sugary, *su su Du Du*.

When ordinary sugary was crossed by amylaceous sugary the F_1 seeds were noticeably wrinkled, but, unlike the true sugary seeds, they were usually, though not always, completely opaque. When the reciprocal cross was made the seeds were usually opaque and smooth, at first glance typical normal starchy seeds. In some cases, however, there was a slight trace of wrinkling. The difference in the appearance of the seeds resulting from reciprocal crosses is undoubtedly a function of the difference in dosage relations in the triploid endosperm,the first cross resulting in a genotype with one dose of su^{am} and two of *Du* while the reciprocal cross yields a genotype with two doses of su^{am} and one of *Du*. But the important fact is that neither cross produced completely starchy seeds. This indicated that one of the genes involved in amylaceous sugary is an allele of ordinary sugary. This conclusion is supported by evidence from linkage tests which show that su^{am} and *su* have approximately the same linkage relations with other genes on the fourth chromosome.

LINKAGE RELATIONS OF su^{am}

An F_1 of sugary-tunicate and amylaceous sugary-nontunicate was crossed by a sugary-tunicate stock. The introduction of the *Du* gene by the backcross parent restricted conspicuous segregation for endosperm texture to the *su*-su^{am} locus. Two classes of seed, translucent and opaque, were produced in approximately equal numbers. When these were planted separately they gave rise to tunicate and nontunicate plants in the following numbers:

Opaque tunicate	94
Opaque nontunicate	187
Translucent tunicate	147
Translucent nontunicate	90

The opaque-tunicate and the translucent-nontunicate plants, which represent crossovers, comprise 35.5 percent of the total. This is approximately the same crossing over (33–36 percent) previously found and reported between *su* and *Tu* in Texas stocks (cf. EMERSON *et al.* 1935).

Other indirect evidence of the allelism of su^{am} and *su* was obtained from linkage tests with the *Ga* and *sp* factors on the fourth chromosome. The *Ga* gene enables the gametes which carry it to accomplish fertilization several times as frequently as those which carry its allele (MANGELSDORF and JONES 1926) and so disturbs Mendelian ratios when visible characters conditioned by other genes on the same chromosome are involved. For example, plants heterozygous for both *su* and *Ga*, when selfed, usually produce 16 percent of sugary seeds instead of the 25 percent which normally occurs in the F_2 endosperm generation of starchy-sugary crosses. If su^{am} is an allele of *su* its segregation should be similarly affected, but here, since a basic 15:1 ratio rather than a 3:1 ratio is involved, approximately four percent (16÷4) of sugary seeds are expected instead of the 6.25 percent which occurs when an undisturbed 15:1 ratio is involved. In a cross of amylaceous sugary with Rice Pop (*Ga Ga*) only 3.1 percent of the seeds (60 in a total of 1911) in the F_2 endosperm generation were sugary. The highly significant deviation from a 15:1 ratio shows that *Ga* is linked with su^{am} and is affecting its segregation.

Corresponding results were obtained in a cross of su^{am} and *sp*, a fourth chromosome gene which reduces the size of the pollen grain. This gene is seldom transmitted through the pollen and on the average only through approximately 42 per cent of the megaspores (SINGLETON and MANGELSDORF 1940). Under Texas conditions plants heterozygous for both *su* and *sp* produce on the average 66.3 percent of sugary seeds. If *su* and su^{am} are allelic the F_2 endosperm generation of the cross su^{am} *du*×*sp* should produce approximately 16.6 percent (66.3÷4) sugary seeds. Actually, 16.1 percent (215 sugary seeds in a total of 1339) occurred.

LINKAGE RELATIONS OF *du*

The *du* factor is located on chromosome 10 and shows linkage with both *R* and *g* on that chromosome.

In progenies homozygous for su^{am} but heterozygous for *du*, segregation for opaque and translucent seeds is approximately 3:1. Two such progenies were encountered in which segregation for aleurone color in a ratio of approximately 3:1 also occurred. The distribution of the four classes was as follows:

Colored opaque	308
Colored translucent	46
Uncolored opaque	83
Uncolored translucent	70

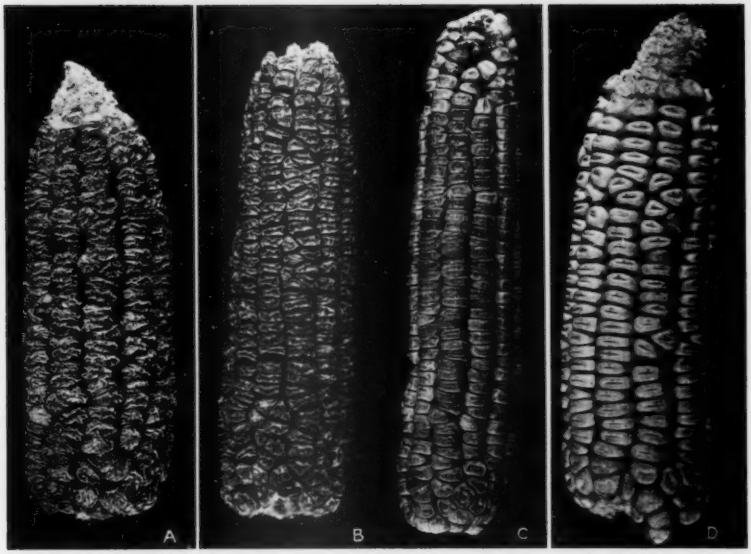

FIGURE 1.—Examples of four true-breeding genotypes which occur in the cross of amylaceous sugary×sugary. A, supersugary (*su su su du du du*); B, sugary (*su su su Du Du Du*); C, amylaceous sugary (su^{am} su^{am} su^{am} *du du du*); D, pseudostarchy (su^{am} su^{am} su^{am} *Du Du Du*).

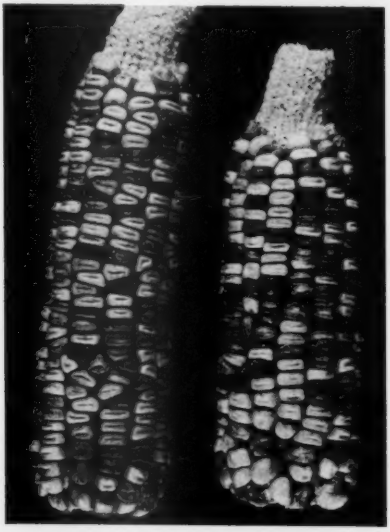

FIGURE 2.—Typical F_2 endosperm segregation in the cross of amylaceous sugary×sugary. The ratio of opaque and translucent seeds is usually 1:1 but may be 9:7 or 7:9.

The distribution suggests linkage with crossing over (computed by IMMER's formula, 1930) of 27.1 percent.

In these two ears it was not known whether segregation for aleurone color was the result of heterozygosity for *R* on chromosome 10 or for *C* on chromosome 9, but further tests demonstrated that it must have been the former since the gene *du* shows independent inheritance with *wx* on chromosome 9 and is definitely linked with *g* on chromosome 10, as shown below.

In the F_2 of a cross of amylaceous sugary × golden in which dull seeds could not be clearly identified, and only the translucent seeds (*su*^{am} *du*) were readily distinguishable from starchy seeds, the following distribution occurred:

Opaque green	462
Opaque golden	187
Translucent green	46
Translucent golden	1

Crossing over between *du* and *g* is computed (IMMER's formula, 1930) at 14 percent.

A three-point test to determine the exact position of *du* on chromosome 10 has not been made. However, since *g* and *R* normally exhibit about 16 percent of crossing over, while *du* in these tests has shown 27 percent crossing over with *R* and 14 percent with *g*, it is probable, though not finally proved, that the order is *R-g-du*.

GENETIC INTERACTION OF *su*^{am}, *du*, AND THEIR ALLELES

The genes *su*^{am} and *du* interact with each other to produce some peculiar and interesting genetic results. When amylaceous sugary, *su*^{am}*du*, is crossed with ordinary sugary, *su Du*, the F_1 seeds, as already noted, are usually opaque and starchy in appearance, though somewhat wrinkled. In the F_2 generation of this cross the segregation seems at first glance to be hopelessly confusing. Variation ranges from seeds which are more translucent and wrinkled than those of the normal sugary parent to smooth, opaque seeds which cannot be distinguished by gross inspection from normal starchy seeds. Nevertheless, the F_2 population can be separated roughly into two classes, one comprising kernels whose general aspect is translucent, the other kernels whose appearance is opaque. When such a separation was made it was found that the two types occurred on the average in approximately equal numbers (2620 translucent seeds in a total of 5317). Individual ears, however, deviated significantly from a ratio of 1:1, some approaching a 9:7 ratio, others a 7:9 ratio. In a population of 15 ears, three with a significant plus deviation produced 55.7 percent of translucent seeds (705 in 1265) while two with significant minus deviations (324 in 775) produced 41.9 percent of translucent seeds.

These deviations are attributable to the fact that there is a more or less definite threshold between the phenotypic condition called translucence and that called opaqueness. Since the endosperm is triploid, there are 16 possible genotypes in the F_2 endosperm generation. The majority of these are phenotypically either opaque or translucent, but at least two genotypes whose pheno-

typic expression lies near the threshold for translucence and opaqueness may appear opaque in some progenies and translucent in others, depending upon differences in the environment or in the genetic background of modifying factors. This threshold, though by no means sharp, is still sufficiently definite so that the borderline genotypes lie clearly to one side or the other,—it does not cut through genotypes but between them. CAMERON, working with isogenic stocks, has shown that other genotypes fluctuate in their phenotypic appearance but in my stocks, at least, there were apparently only two which regularly crossed the threshold from translucence to opaqueness or vice versa. CAMERON also finds that the typical ratio among isogenic genotypes (resulting from repeated backcrossing to inbred I 45) is 9:7 rather than 1:1 with respect to opaqueness and translucence. It is easily conceivable, however, that isogenic stocks derived by backcrossing to other inbreds would regularly yield 1:1 or 7:9 ratios.*

In the F_2 generation two new true breeding types, *su du* and *su^{am} Du*, appeared. The first is extremely translucent and wrinkled and since it shows in exaggerated form the characteristics which distinguish sugary from starchy it is called "supersugary." The second new type is scarcely distinguishable from normal starchy and is called "pseudostarchy," a term previously used by JONES (1919) to describe a condition genetically different from starchy but phenotypically difficult to distinguish from it. The pseudostarchy condition described here, however, has quite a different origin than that reported by JONES and it is also genetically different since it involves a new allele of *su*.

The results obtained in the F_2 and F_3 generations of the sugary-amylaceous sugary cross and results of additional tests to be reported later in this paper show the genetic constitution of normal starchy and of the four true breeding types isolated from the cross to be as follows:

Starchy	*Su Su Du Du*
Pseudostarchy	*su^{am} su^{am} Du Du*
Amylaceous sugary	*su^{am} su^{am} du du*
Sugary	*su su Du Du*
Supersugary	*su su du du*

The results of additional tests which verify these conclusions are set forth below. The description of the triploid endosperm genotypes occurring in the various crosses conforms with the shorthand notation which is used in CAMERON'S paper. The number of doses of *su^{am}* and *Du*, respectively, in the 16 possible triploid genotypes is indicated by two numbers separated by a dash. The genotypes vary from supersugary, *su su su du du du*, which is called genotype 0-0 to pseudostarchy, *su^{am} su^{am} su^{am} Du Du Du*, which is called genotype 3-3.

(Genotype 3-3×Genotype 0-3) F_2

F_1 seeds of this cross (genotype 2-3) were completely opaque but definitely more wrinkled than the pure pseudostarchy kernels. This indicates that the

* Since this was written I have found that the typical F_2 ratio in a stock backcrossed twice to inbred P 39 is 7 opaque: 9 translucent.

su^{am} gene is not so completely dominant over *su* as is *Su*, the third allele of the series.

In the F_2 generation of this cross only *su* and *su*^{am} are segregating, since the population is constant for *Du*. Four endosperm genotypes having 0, 1, 2, or 3 doses of *su*^{am} (genotypes 0-3, 1-3, 2-3, and 3-3) are expected. The Mendelian ratio on these ears was a normal 3:1 (173 translucent seeds in a total of 603). It is obvious from these results that in the presence of three doses of *Du*, one dose of *su*^{am} is adequate to produce an opaque condition not readily distinguishable from true starchy.

(Genotype 3-3×Genotype 0-3) F_1×Genotype 0-3

When the heterozygote whose F_2 is described above was backcrossed by sugary, two triploid genotypes, 0-3 and 2-3, are expected in approximately equal numbers. The first was translucent and the second, opaque. Observed numbers were 166 translucent:156 opaque.

Genotype 0-3×(Genotype 3-3×Genotype 0-3) F_1

In this backcross, the reciprocal of that described immediately above, a 1:1 segregation is again expected, but here there should be less difference in the two genotypes since one is 0-3 and the other, 1-3, and the only difference between them is in one dose of *su*^{am}. The two genotypes were somewhat less distinct than in the reciprocal backcross but were still readily distinguishable. Numbers observed were 830 opaque:773 translucent. This is further proof that one dose of *su*^{am} in the presence of three doses of *Du* can change translucence to opaqueness.

Genotype 3-0×(Genotype 3-3×Genotype 0-3) F_1

The two genotypes expected from this cross are 3-1 and 2-1. Both genotypes should be opaque in appearance but the second might be expected to be slightly more wrinkled than the first. Only one ear of this cross was obtained. It exhibited definite segregation in the degree of wrinkling but an accurate classification of the individual seeds was impossible.

Genotype 3-0×(Genotype 0-0×Genotype 0-3) F_1*

The two genotypes, 2-0 and 2-1, are expected from this pollination. The first produced translucent seeds; the latter, opaque seeds. Actual numbers were 477 translucent:444 opaque. This cross is of particular interest because the backcross parent and both of the genotypes involved in the F_1 as well as the F_1 itself have translucent seeds. Yet the interaction of the two genes gives rise to a clear-cut segregation for opaque and translucent seeds. This cross is also of interest in showing that one dose of *Du* can produce opaqueness in the presence of two doses of *su*^{am}.

* The identity of this genotype was not definitely known when the cross was made but was deduced from the appearance of the F_1 and the breeding behavior in backcrosses.

Genotype 3-0×(Genotype 0-0×Genotype 3-0) F_1*

This cross should give rise in equal numbers to the two genotypes, 2-0 and 3-0. Both are translucent and the two types differ only in degree. An ear of this cross exhibited segregation for the degree of wrinkling and translucence but an accurate classification was not possible.

(Genotype 0-0×Genotype 3-0) F_1×Genotype 0-3*

This pollination should give rise to genotypes 0-1 and 2-1. Two types were obtained, one with strongly translucent and wrinkled kernels and the other with opaque slightly wrinkled kernels. Actual numbers were 242 opaque:203 translucent. This represents a second example of crossing a translucent genotype with an F_1 of two translucent genotypes to obtain a 1:1 segregation for opaque and translucent kernels.

CHEMICAL COMPOSITION AND POSSIBLE HORTICULTURAL VALUE OF TRUE BREEDING GENOTYPES

Through the cooperation of DR. GLENN A. GREATHOUSE, chemical analyses of the four true breeding types were obtained. These are set forth in table 1.

TABLE 1

Chemical composition of entire seeds of four genotypes occurring in the cross, Amylaceous Sugary×Sugary.

DESCRIPTION	GENOTYPE	NO. SAMPLES	PERCENT					
			REDUCING SUGARS	SUCROSE	TOTAL SUGARS	DEXTRINS	STARCH	FAT
Supersugary	0-0	2	1.74	3.44	5.44	18.40	27.99	7.20
Sugary	0-3	2	1.60	2.68	4.50	13.10	40.17	5.42
Amylaceous Sugary	3-0	2	.87	2.14	3.16	4.80	45.02	5.02
Pseudostarchy	3-3	1	0.51	0.85	1.46	0.34	56.19	4.53

The stocks upon which these chemical analyses were made were not isogenic and the data derived from them cannot be used for reaching precise conclusions regarding the interaction of the genes involved. This is an important aspect of the problem which is treated in detail in a separate paper by CAMERON. The data included here, however, reveal that both *su^{am}* and *du* bring about an increase in starch and total carbohydrates and a concomitant decrease in dextrins and sugars.

Two rather definite conclusions can be drawn from the data: 1) that *su^{am}* has a greater effect than *Du;* 2) that the combined effects of *su^{am}* and *Du* are at least equal to the sum of their separate effects. From other facts previously mentioned, a third conclusion can be drawn; that successive doses of the same gene are not additive. For example, genotypes 2-1 and 1-2 are both opaque

though sometimes slightly wrinkled. This shows that three doses of the two genes acting in combination are capable of producing opaqueness. Yet the genotypes 3-0 and 0-3 are always translucent which shows that three doses of either gene acting alone are not capable of producing opaqueness. If successive doses of the same gene were additive, then genotype 3-0 with three doses of *su*^{am}, the more effective of the two genes, should be more strongly opaque than the genotype 1-2 with only one dose of *su*^{am} and two doses of *Du*. The contrary is true.

These facts are of considerable theoretical interest as CAMERON'S much more extensive data show clearly. The preliminary data included here are of importance primarily in showing the general nature of the gene interaction and in pointing out the possibilities of breeding new sweet corn varieties having chemical compositions different from those now on the market.

It is well known that quality in sweet corn, as distinguished from starchy or field corn, is correlated with the percentage of water-soluble polysaccharides in the kernel at the roasting ear stage, for it is these components which give sweet corn its sweetness and "creaminess." With appropriate combinations of true breeding genotypes derived from crosses of amylaceous sugary, it should be possible to produce, within the range of the known extremes, almost any percentage of water-soluble polysaccharides which might be desirable. Supersugary, the most extreme true breeding form, contains appreciably higher amounts of sugars and dextrins than common sweet corn and it may prove to be useful under some conditions. This true breeding type, however, is also more susceptible than ordinary varieties of sweet corn to molding in the fields while maturing and it may not be wholly satisfactory as a variety or F_1 hybrid. A "blend" intermediate between supersugary and sugary can be easily produced by crossing an inbred strain of sugary with an inbred strain of supersugary. Ears of the F_1 hybrid will bear four endosperm genotypes having 0, 1, 2, and 3 doses, respectively, of *Du* in approximately equal numbers. These will not be distinguishable at the roasting ear stage, for they are scarcely distinguishable in the mature kernels, but their combined product should have a chemical composition that is appreciably higher in water-soluble polysaccharides than the sweet corn varieties now used commercially.

In the other direction, true breeding amylaceous sugary may have some usefulness in regions such as the Southwest where ordinary sugary is handicapped, first by poor germination in the spring, and later by greater susceptibility than ordinary field corn to the effects of heat and drought. Amylaceous sugary is intermediate between true sugary which is not wholly satisfactory from the standpoint of productiveness, and field corn varieties which are extensively grown in the Southwest for roasting ears, but whose table-quality leaves much to be desired.

There is one additional horticultural use to which amylaceous sugary can probably be put. It can be used as a simple tester for the modifier complex affecting the chemical composition of inbred strains of sweet corn. It has already been pointed out that several of the 16 possible endosperm genotypes may fluctuate in their external appearance as the result of differences in the back-

ground of modifiers. Two of the genotypes which fluctuate in this way are genotype 2-1 which results when amylaceous sugary is pollinated by sugary, and genotype 1-2 which results from the reciprocal cross. By crossing an inbred strain of amylaceous sugary with a group of inbred strains of sweet corn differences in the degree of wrinkling and translucence of the F_1 seeds should reflect differences in the modifier complexes of the strains tested. And the test has possibilities of being a very sensitive one, for inbred strains of sweet corn which in external appearance are almost identical may still differ sufficiently in their modifier complex to tip the scales when that complex is acting upon a genotype near the threshold between translucence and opaqueness.

Preliminary tests involving the sweet corn inbreds P 39, I 45, and Conn. 13 indicate that they rank in that order with respect to desirable modifier complexes.

It is not yet certain whether the genotype 1-2 resulting from the cross sugary × amylaceous, or 2-1 resulting from the reciprocal cross, is the better as a tester. Genotype 1-2 is more sensitive to fluctuations than genotype 2-1 but there is an advantage in having all test-crosses made on, rather than by, the same inbred strain.

THE GENETIC COMPOSITION OF SEVERAL SWEET CORN VARIETIES WITH RESPECT TO *su*^{am} AND *du*

Since varieties of sweet corn similar to the one which STURTEVANT described as *amyleasaccharata*, and hence also similar to amylaceous sugary, have been reported from Mexico, the Southwest, and Peru (STURTEVANT 1899), and since a condition approaching amylaceous sugary has been described in prehistoric corn (HENDRY 1930), it is reasonable to suspect that some of the sweet corn varieties grown by man in prehistoric and present times may, like the amylaceous sugary described in this paper, involve the *su*^{am} allele rather than the more common *su* allele. Three varieties whose appearance is similar to amylaceous sugary, Papago sweet from Arizona, and two varieties from Bolivia, were crossed with both sugary and amylaceous sugary. All proved to be *su Du*, although all were distinctly more starchy in appearance than the common commercial sweet corn varieties in the United States.

It was also suspected that some of the sweet corn varieties in the United States which are recognized as having especially good quality might have the gene *du* which in combination with *su* is known to produce the condition described as supersugary. Crosses of amylaceous sugary with Purdue 39, Country Gentlemen, Black Mexican, Narrow Grain Evergreen and Golden Colonel show that all of these varieties are of the genetic composition *su su Du Du*.

There is, therefore, no evidence that amylaceous sugary has ever been used by man as a pure variety or that the gene *du* enters into the composition of high-quality sweet corn varieties. The gene *du*, however, seems not to be uncommon for it has been discovered in several additional Southwestern varieties. The frequency of *su*^{am} is not known for there is no way of determining, without extensive genetic tests, how commonly the *su*^{am} gene is distributed among field

corn varieties, since its presence is not apparent in the absence of *du*. It was apparently only the fortuitous presence of the two genes in combination which led to the discovery of amylaceous sugary in the Surcropper variety.

DISCUSSION

Amylaceous sugary, and its derivatives from crosses with sugary, furnish an especially excellent example of the intergradation of qualitative and quantitative inheritance. The two genes involved are producing approximately the same effect; chemically, an increase in starch; phenotypically, an increase in opaqueness. They are accomplishing this more or less independently of each other since the combined effects of the two are at least additive. Inheritance is quantitative in the sense that the different genotypes differ from each other primarily in degree; it is qualitative in the sense that in appropriate backcrosses the segregation of two different genotypes is usually clear-cut and their frequency conforms to simple Mendelian ratios.

SUMMARY

1. Amylaceous sugary is a new type of sugary endosperm isolated by inbreeding from a Southwestern field corn variety.

2. Amylaceous sugary involves two genes, su^{am}, an allele of *su*, and *du*, which, acting alone, produces a dull appearance in the seeds.

3. The gene su^{am} shows approximately the same linkage relations with the genes, *Tu*, *Ga*, and *sp*, on the fourth chromosome, as does *su*.

4. The gene *du* is located on chromosome 10 where it shows approximately 27 percent of crossing over with *R* and 14 percent with *g*. The presumed order is *R-g-du*.

5. From crosses of sugary, *su Du*, and amylaceous sugary, su^{am} *du*, two new true-breeding genotypes, supersugary, *su du*, and pseudostarchy, su^{am} *Du*, can be isolated.

6. The four true-breeding genotypes isolated from this cross differ in chemical composition and show that both su^{am} and *Du* affect the percentages of sugars, starches and dextrins and that their combined effect is at least as great as the sum of their separate effects. Successive doses of the same gene, however, are not additive.

7. Crosses of sugary and amylaceous sugary by starchy-appearing sweet corn varieties from Arizona and Bolivia show that these do not have the su^{am} allele, and hence are not amylaceous sugary.

8. Crosses of amylaceous sugary by high-quality commercial sweet corn varieties show that none of those tested have the gene *du*.

9. Sweet corn varieties and inbreds can be easily improved with respect to content of water-soluble polysaccharides by substituting the gene *du* for *Du*.

10. Crosses of an inbred strain of amylaceous sugary with sugary can probably provide a simple method of testing sweet corn inbreds for modifier complexes which affect quality.

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CHEMICO-GENETIC BASES FOR THE RESERVE CARBOHYDRATES IN MAIZE ENDOSPERM[1]

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INTRODUCTION

THE relationships between genes and metabolic processes have received increasing attention in recent years. Morphological studies have been supplemented by chemical and biochemical investigations which attempt to discover the physiological bases of gene action. Examples include the findings on genetic control of the flower pigments in Primula, Dahlia, *etc.* (summarized by SCOTT-MONCRIEFF, 1936); the studies by WRIGHT (*e.g.*, 1941) on the interactions of pigment factors in the guinea pig; and especially the researches of BEADLE, TATUM, and associates (see HOROWITZ *et al.*, 1945) on amino acid and vitamin synthesis in Neurospora. Such studies have revealed genic control over single, specific chemical reactions. While other genetic analyses appear to indicate greater complexity, this is due at least in part to the levels at which the investigations are made and may only mean that groups of interactions have not yet been separately defined.

The present study represents one of these cases, being concerned with gene action as deduced from the examination of the carbohydrates stored in maize endosperm. Differences which are conditioned by two pairs of alleles, and a possible interpretation in terms of starch-synthesizing enzymes of the phosphorylase system are considered.

Genes affecting the endosperm carbohydrates

The endosperm properties called "sugary" and "starchy" are primarily conditioned by the gene su_1 and its normal allel Su_1 (CORRENS, 1901; EAST and HAYES, 1911). Kernels homozygous for su_1 are translucent, wrinkled, and glassy in texture, while Su_1 kernels are opaque, smooth, and starchy in appearance. The gene pair behaves in simple mendelian fashion, with Su_1 exhibiting essentially complete dominance. While the term starchy quite accurately describes the endosperm types to which it refers, sugary kernels differ at maturity not primarily by their higher content of simple sugars, but in that a large fraction of their reserve carbohydrate occurs as water-soluble polysaccharides rather than as granular starch.

Modifying factors for the Su_1su_1 system were described by JONES (1919), who developed strains which he called pseudostarchy by selection among individuals homozygous for su_1. Pseudostarchy kernels simulated the Su_1 condition in outward appearance, but progeny tests indicated that the funda-

[1] This study was carried out at the BIOLOGICAL LABORATORIES, HARVARD UNIVERSITY, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

mental properties of the su_1 gene had remained unchanged. JONES concluded that independently inherited modifying factors in the plant and endosperm were probably responsible for the effects observed.

EYSTER (1934) reported a recessive gene su_2 (located on chromosome VI and thus independent of su_1 on chromosome IV) which when homozygous causes varying degrees of endosperm translucence and occasionally some wrinkling. It is thus less marked in its action than su_1. HOROVITZ, MARCHIONI, and FISHER (1941) described the gene "sugary-x" (su_x), also on chromosome VI. Its effects in combination with homozygous Su_1 were similar to those of su_2 but linkage studies suggested that the two were separate genes. However, additional studies by C. R. BURNHAM (personal communication) indicate that su_x may be identical to su_2.

The gene waxy (*wx*) first described by COLLINS (1909) is so called because of the dull, waxy appearance it imparts to starchy endosperms. The principal visible evidence of its effects on the carbohydrates is the red-brown color reaction of waxy starch with iodine, which depends upon the fact that this starch is entirely of the branched-chain variety, amylopectin. (BATES, FRENCH, and RUNDLE, 1943; SPRAGUE, BRIMHALL, and HIXON, 1943).

Two additional genes called amylaceous (su_1^{am}) and dull (*du*) are the basis for the present study. Their mode of inheritance has been demonstrated by MANGELSDORF (1947) who showed that su_1^{am} is an allel of su_1 while *du* on chromosome X is independently inherited. In addition to the normal sugary genotype su_1su_1 *Du Du* he obtained three new true-breeding types, $su_1^{am}su_1^{am}$ *du du* (amylaceous sugary), $su_1^{am}su_1^{am}$ *Du Du* (pseudostarchy), and su_1su_1 *du du* (supersugary) as well as intermediate types, and showed that the interaction of su_1^{am} with *Du* results in essentially starchy-type endosperm.

Previous evidence on the changes in carbohydrate fractions which accompany gene changes

Several studies which have been made upon the chemical composition of the endosperm carbohydrates are of interest to the present work. PEARL and BARTLETT (1911) followed the inheritance of sugars and starch in crosses involving sweet and starchy corn. They reported more sugars and less starch in the mature kernels of sweet types than in starchy ones.

JONES (1919) obtained analyses of mature samples of his sugary, starchy, and pseudostarchy kernel types. The content of soluble carbohydrates was high in the sugary kernels and low in the starchy ones. The pseudostarchy types were nearly as low in this fraction as the true starchy ones.

CULPEPPER and MAGOON (1924) and BERNSTEIN (1943) analyzed developing kernels of several lines of sweet and starchy corn, conditioned by the genes *su* and *Su*. (From here on the subscript for this allelic series is omitted.) In sugary types the rate of starch accumulation was low, and was accompanied by an increase in dextrins; in starchy types the starch content rose more rapidly, and only small quantities of dextrins were present at any stage.

LINDSTROM and GERHARDT (1926, 1927) using mature kernels, measured the water-soluble and insoluble carbohydrates in the parental types, F_1 hybrids,

and some succeeding generations of sugary and starchy lines of maize. The ratio of soluble to insoluble components was about 2:1 in most sugary types and usually less than 1:10 in starchy ones.

BRINK and ABEGG (1926) comparing mature, non-sugary kernels of waxy and non-waxy corn, showed that in both of these genetic types the starch content was high, and that dextrins were correspondingly low.

ANDREW, BRINK, and NEAL (1944) studied the behavior of sugars and water-soluble polysaccharides during kernel development, in four types conditioned by the genes *Su Wx*, *su Wx*, *Su wx*, and *su wx*. In each genotype, total sugars were highest at the first sampling date (12 days after pollination) and decreased sharply during development. The sugary types maintained a higher percent both of sugars and of soluble polysaccharides than did the starchy ones, and within these two classes waxy kernels showed slightly higher values than non-waxy.

Several of the papers just cited show that the starch grains from starchy and sugary endosperms differ markedly in form. Starchy kernels contain for the most part, large, simple grains while in sugary kernels small, angular bodies of starch appear in aggregates called compound grains. Starch grain form is little affected by the waxy gene, however, either in mature kernels (BRINK and ABEGG, 1926) or in developing grain (LAMPE, 1931).

The structure and biochemistry of starch

Important advances have been made in recent years in the knowledge of the structure and biochemistry of starch and glycogen. (See MEYER, 1942; HASSID, 1945; CORI, SWANSON, and CORI, 1945). Most plant starches contain two components, amylose and amylopectin, whose properties are widely different. Amylose, which is considered to be an essentially straight-chain molecule of perhaps two hundred glucose units, is responsible for the blue staining reaction of starch with iodine. Amylopectin is of much higher molecular weight, is composed of many short glucose chains in branching arrangement, and gives a red-brown color with iodine. Waxy maize starch and a few other naturally occurring starches are exclusively amylopectin. The animal polysaccharide, glycogen, is similar to amylopectin, but shows some differences in the nature of its branching.

A class of enzymes known as phosphorylases has been shown to be concerned with the reversible synthesis of both glycogen (CORI, COLOWICK, and CORI, 1938, 1939; CORI, SCHMIDT, and CORI, 1939) and starch (HANES, 1940a, 1940b; BOURNE and PEAT, 1945; BOURNE, MACEY, and PEAT, 1945). The enzymes have been found in a wide variety of tissues including liver, heart, and brain; and peas, potatoes, Lima beans, and waxy maize. (For the latter case see BLISS and NAYLOR, 1946.) Potato phosphorylase brings about the formation of a polyglucose having the properties of amylose and the same action is characteristic of purified muscle phosphorylase. The synthesis of amylopectin and glycogen, however, requires the collaboration of at least one related enzyme, which has been called "Q enzyme" or "branching factor."

Experiments of interest in relation to the developments outlined above have

recently been reported for the water-soluble polysaccharide fraction of sweet corn. MORRIS and MORRIS (1939) separated from this material a subfraction which they called "corn glycogen." HASSID and MCCREADY (1941) studied the subfraction and found that it had many of the properties of glycogen. SUMNER and SOMERS (1944) separated the water-soluble polysaccharides into two subfractions. One of these was "corn glycogen," which they renamed "phytoglycogen"; the other they called "glycoamylose." Phytoglycogen stained brown with iodine, while glycoamylose stained blue although not with the intensity of amylose.

MATERIALS AND METHODS

Production of the genetic material

In the triploid endosperm of maize, a series of sixteen genotypes is available by the use of the two pairs of alleles *su*$^{a^m}$*su*, and *Du du*. To obtain this series a Texas strain of amylaceous sugary was crossed with the normal sugary inbred, Iowa 45. The F_1 hybrid was twice backcrossed to the Iowa 45 parent and the resulting plants were selfed. Further generations of selfing permitted the isolation of each of the four pure-breeding genotypes. These were grown side by side in the summer of 1945 and were selfed and intercrossed in all possible combinations. The ears were allowed to mature fully in the field and were stored in the air-dry condition until used. Kernels descended from three times backcrossed material have also recently been obtained and although not analyzed chemically, resemble closely those of their countertypes in the twice backcrossed stock.

Methods of chemical analysis

For carbohydrate analyses kernels from three to six ears of the same genotype were pooled. Samples weighing 15.0 grams were taken and the embryos removed with a knife point. The remaining tissue is hereafter referred to as endosperm, although the thin, maternal pericarp was still present. The endosperms were ground to 80 mesh in a Wiley electric mill, a sample was immediately removed for moisture determination, and the remainder of the tissue (10 to 12 grams) was weighed into a Whatman extraction thimble. Sugars were removed by extracting for six to eight hours in a Soxhlet apparatus with 80 percent ethanol. Reducing and non-reducing sugars were determined by the method of HASSID (1936). Difco Invertase solution was regularly employed in the determination of sucrose. Several comparisons of results obtained by the use of HCl indicated that there was no difference in the amount of reducing substances formed by the two methods. Duplicate titrations for both reducing sugars and sucrose, made on each sample, ordinarily agreed within two to three percent.

For the determination of total water-soluble polysaccharides and subfractions thereof, a method similar to that described by SUMNER and SOMERS (1944) was used. The dried residue from the sugar extraction was pulverized, transferred quantitatively to a flask and shaken with 60 cc. of aqueous, ten percent trichloracetic acid for one-half hour. The suspension was separated in

a Sorvall supercentrifuge, and the residue twice reextracted in the same manner. Tests indicated that more than 95 percent of the water-soluble polysaccharides were removed by three extractions, from even the most sugary types of tissue. The combined extracts were precipitated by the addition of two volumes of 95 percent ethanol, the precipitate washed twice with ethanol, and dried to constant weight at 70°C *in vacuo*. This material is reported as total water-soluble polysaccharides. In order to avoid gross temperature changes which might have affected the amounts of polysaccharide dissolved, the extractions were carried out in a 17°C, constant temperature room.

The residue was dried at 50°C, weighed, and then reground to a uniform mixture. Duplicate 1.0 gram samples were taken for starch determination and simultaneously a sample was taken for moisture determination. Starch was determined by the method reported by BRIMHALL and HIXON (1945).

The experimental error of the values obtained by these carbohydrate analyses is probably high. This is the case, not primarily as a result of technical errors, but because of the nature of the experimental material. The particular quantities of sugars, water-soluble polysaccharides, and starch which occur in the members of such a series are subject to environmental modification, as will be evident from the fluctuation to be described in the appearance of many of the endosperm types. Complete duplicate fractionations were obtained for only a few of the genotypes, although the general reproducibility of results was clear from preliminary trials. Results of assays on such duplicates varied from three to fifteen percent.

Granular starch for the determination of the amylose: amylopectin ratios was isolated from duplicate samples of kernels drawn from the pools described above. The method has been published by BRIMHALL, SPRAGUE, and SASS, (1945). Potentiometric iodine titration (BATES, FRENCH, and RUNDLE, 1943) was employed to determine the percentage of amylose present; amylopectin was then derived by difference. For this property the agreement of duplicates was usually within five percent. This work was carried out at the Department of Chemistry, Plant Chemistry Subsection, IOWA STATE COLLEGE, Ames, Iowa.

When total water-soluble polysaccharides were to be obtained by direct extraction from whole kernel tissue (SUMNER and SOMERS, 1944) the kernels were soaked overnight in distilled water with toluene, and then macerated for two to three minutes in a Waring blender with cold, ten percent trichloracetic acid. The suspension was centrifuged and the residue four times reextracted, after which the soluble polysaccharides were precipitated from the supernatant by the addition of two volumes of 95 percent ethanol.

The SUMNER and SOMERS method was also employed in the subfractionation of the water-soluble polysaccharides. One gram samples were dissolved in ten ml of distilled water. Glycoamylose was precipitated with two volumes of glacial acetic acid and phytoglycogen was recovered from the supernatant by the addition of 1.5 volumes of 95 percent ethanol. The glycoamylose fraction was redissolved and reprecipitated and the additional material extracted was recovered as before, by the addition of ethanol. The subfractions were then washed in ethanol and their dry weights determined. The weight of sample used

TABLE 1

The gene dosages, phenotypes, and quantities of various carbohydrates† in the 16 endosperm genotypes resulting from combinations of the two gene pairs, $su^{am}su$, and Du du.

GENOTYPE	DOSES PRESENT su^{am} Du	DESCRIPTION OF PHENOTYPE	REDUCING SUGARS	SUCROSE	TOTAL SUGARS	TOTAL WATER-SOLUBLE POLYSACCHARIDES	STARCH	TOTAL CARBOHYDRATES	AMYLOPECTIN IN GRANULAR STARCH
			%	%	%	%	%	%	%
$su^{am}su^{am}su^{am}su^{am}$ *DuDuDu*	3-3	Opaque, smooth	0.05	0.03	0.08	0.4	80.7	81.2	68.7
$su^{am}su^{am}su^{am}su^{am}$ *DuDudu*	3-2	Opaque, smooth	0.04	0.05	0.09	0.4	77.7	78.2	68.2
$su^{am}su^{am}su$ *DuDuDu*	2-3	Opaque, smooth	0.06	0.06	0.12	1.0	76.0	77.1	68.0
$su^{am}su^{am}su$ *DuDudu*	2-2	Opaque, smooth*	0.06	0.07	0.13	1.9	79.6	81.6	68.7
$su^{am}su^{am}su^{am}su^{am}$ *Dududu*	3-1	Opaque, smooth*	0.05	0.06	0.11	0.6	72.7	73.4	68.2
$su^{am}su^{am}su$ *Dududu*	2-1	Nearly opaque, slightly wrinkled*	0.06	0.18	0.24	1.7	71.8	73.7	68.6
$su^{am}susu$ *DuDuDu*	1-3	Opaque, smooth*	0.11	0.12	0.23	1.8	69.7	71.7	69.8
$su^{am}susu$ *DuDudu*	1-2	Nearly opaque, slightly wrinkled*	0.14	0.16	0.30	4.1	70.3	74.7	68.9
$su^{am}susu$ *Dududu*	1-1	Semi-opaque, partially wrinkled*	0.14	0.28	0.42	14.2	58.6	73.2	67.8
$su^{am}su^{am}su^{am}su^{am}$ *dududu*	3-0	Translucent, wrinkled, pale orange	0.30	0.52	0.82	13.5	51.5	65.8	50.0
$su^{am}su^{am}su$ *dududu*	2-0	Translucent, wrinkled, pale orange	0.27	0.62	0.89	17.8	42.0	60.7	48.9
$su^{am}susu$ *dududu*	1-0	Translucent, wrinkled, pale orange	0.23	0.71	0.94	25.9	33.6	60.4	43.6
sususu *DuDuDu*	0-3	Translucent, strongly wrinkled, yellow	1.53	1.10	2.63	32.8	32.2	67.6	61.4
sususu *DuDudu*	0-2	Translucent, strongly wrinkled, yellow	1.10	1.12	2.32	32.2	30.0	64.5	59.6
sususu *Dududu*	0-1	Translucent, strongly wrinkled, yellow	1.81	1.16	2.97	35.9	27.2	66.1	57.8
sususu *dududu*	0-0	Translucent, strongly wrinkled, yellow	1.39	1.20	2.59	40.8	19.2	62.6	35.0

* These genotypes can fluctuate in appearance. See text for further description.

† Expressed as percents of mature endosperm tissue, on a dry weight basis.

for this subfractionation was chosen for technical reasons. Its smallness resulted in losses during handling, which totaled about ten percent. The losses are believed to have been randomly distributed and should have little bearing on the results.

Enzyme extracts of developing kernels were prepared as described by BLISS and NAYLOR (1946). The fraction precipitated once at a concentration of 36 grams of ammonium sulfate per 100 ml of extract was used. Reaction mixtures for incubation were prepared according to the method of GREEN and STUMPF (1942). Phosphorus was determined colorimetrically using a Beckman spectrophotometer at a wave length of 390 mμ (SPERRY, 1942).

EXPERIMENTAL RESULTS

The data relative to the principal carbohydrate fractions and a descriptive tabulation of the appearance of the endosperm types appear in table 1. No single arrangement of the genotypes to make clear all the relationships was possible; therefore an arbitrary order was adopted, based first upon a separation into grossly sugary-type and starchy-type endosperm, and second upon increasing dosages of the two higher alleles (su^{am} and *Du*) separately, each on backgrounds constant for the other.

To facilitate reference to any genotype, a notation indicating the number of the higher alleles present was adopted and appears in the second column of the table. For example, the notation 2-1 means that two doses of su^{am} and one dose of *Du* are present and, therefore, one dose of *su* and two of *du*. The gene dosages so represented appear in the first column.

Description of the endosperm phenotypes

Seven of the 16 genotypes regularly fall into the class referred to above as sugary-type. They are 0-0, 0-1, 0-2, 0-3, 1-0, 2-0, and 3-0, and they appear in that ascending order (table 1). The nine genotypes which lie above them, 1-1, 1-2, 1-3, 2-1, 3-1, 2-2, 2-3, 3-2, and 3-3, form the starchy-type group. It should be noted that all 16 types are homozygous for the yellow endosperm gene Y_1. Therefore, the orange hue described below must be considered to be related to the gene su^{am}. Genotypes 0-0, 0-1, 0-2, and 0-3 form a group constant for zero doses of su^{am} (*i.e.*, homozygous for *su*) and containing respectively zero, one, two, and three doses of *Du*. They are essentially homogeneous in appearance, being translucent, strongly wrinkled, and yellow. The effects of *Du* are in these respects scarcely discernible within the group, even though two different pure-breeding types, supersugary (0-0) and normal sugary (0-3), are included. Occasionally the kernels of supersugary are slightly more wrinkled and paler than the others. Ears of these four genotypes are shown as a group in Plate I, figure A.

A second group of genotypes of homogeneous appearance includes 1-0, 2-0, and 3-0. The last member of this group is the pure-breeding type, amylaceous sugary. Although definitely sugary-type, with glassy tissue lacking extensive starch-packed areas, the kernels of this group differ from those of the first in two notable respects. They are less wrinkled, generally displaying a single,

large surface depression on the cap, but having smooth, plump side walls with but few auxiliary wrinkles; and their color is a pale, dull orange. This color while not radically different from the yellow of the first group, has been constant in the stock used and has made possible the accurate separation of the three genotypes bearing it, from the other sugary types. A complete dosage series is formed when supersugary (o-o) is again used as a point of departure giving the sequence o-o, 1-o, 2-o, and 3-o. The addition of one or more doses of *su*^{am}, however, changes the phenotype considerably more than doses of *Du*. This sequence of genotypes is shown in Plate I, figure B. The photograph does not reveal the color difference, but the higher degree of wrinkling on the supersugary ear is easily visible.

The next genotype in ascending order in table 1 is 1-1. At this point the starchy-type threshold is passed and changes in endosperm appearance in the nine following genotypes are less easy to differentiate. Genotype 1-1 regularly shows intermediate characters, being usually partially wrinkled and semi-opaque. It can fluctuate in the direction of starchiness sufficiently to give entire ears with nearly smooth kernels, but has never been found to fall into the true sugary-type class. The two shades of color, so definitive for the sugary-type groups, are not consistently maintained in the starchy group.

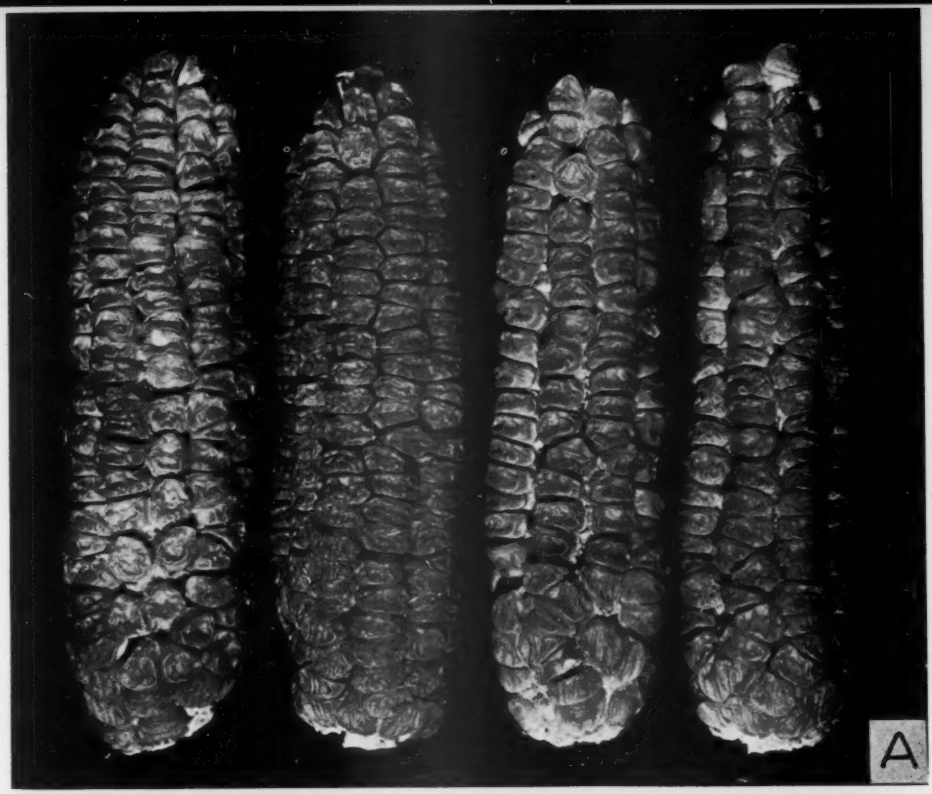
With genotype 1-1 as a point of departure, the effects of increasing dosages of *su*^{am} and *Du* may again be examined. Genotypes 1-2 and 1-3 show added increases in starchiness. Genotype 1-2 is commonly nearly opaque and only very slightly wrinkled, while 1-3 is usually opaque and smooth. Both genotypes can fluctuate from ear to ear and upon the same ear, so that it is not possible to identify them among a group of segregating kernels. A similar situation exists as regards genotypes 2-1 and 3-1. An increase in starchiness, on the average, accompanies an increase in *su*^{am} genes, but the degree of starchiness attained is not constant. These two groups of genotypes, together with the two appropriate sugary-type ears necessary to form the two series, 1-o, 1-1, 1-2, 1-3, and o-1, 1-1, 2-1, 3-1 are illustrated in Plate II, figures A and B. An ear of genotype 1-1 appears second from the left in each figure. Two ears with different degrees of wrinkling were chosen to show the fluctuation possible. Unfortunately the photograph cannot make clear that the kernels on the ear in figure B, while essentially smooth, are still only semi-opaque.

Of the remaining four genotypes in table 1, only one has ever shown any fluctuation. This is genotype 2-2, which is usually completely opaque and smooth, but occasionally exhibits small semi-starchy areas. The other three, 2-3, 3-2, and 3-3 are consistently opaque and smooth, the last one being the pure-breeding type, pseudostarchy. It appears that as far as external appearance is concerned, the phenotypes resulting from these combinations of genes are beyond the influence of modifying conditions which can affect the intermediate genotypes. The four true-breeding genotypes, o-o, o-3, 3-o, and 3-3 are particularly interesting. Selfed ears of these are shown as a group in Plate III.

PLATE I

FIGURE A.—The four endosperm genotypes o–o, o–1, o–2, and o–3 (from left to right). All are sugary-type and are almost indistinguishable from one another in appearance.

FIGURE B.—The four endosperm genotypes o–o, 1–o, 2–o and 3–o (from left to right). All are sugary-type but genotype o–o is more highly wrinkled than the others.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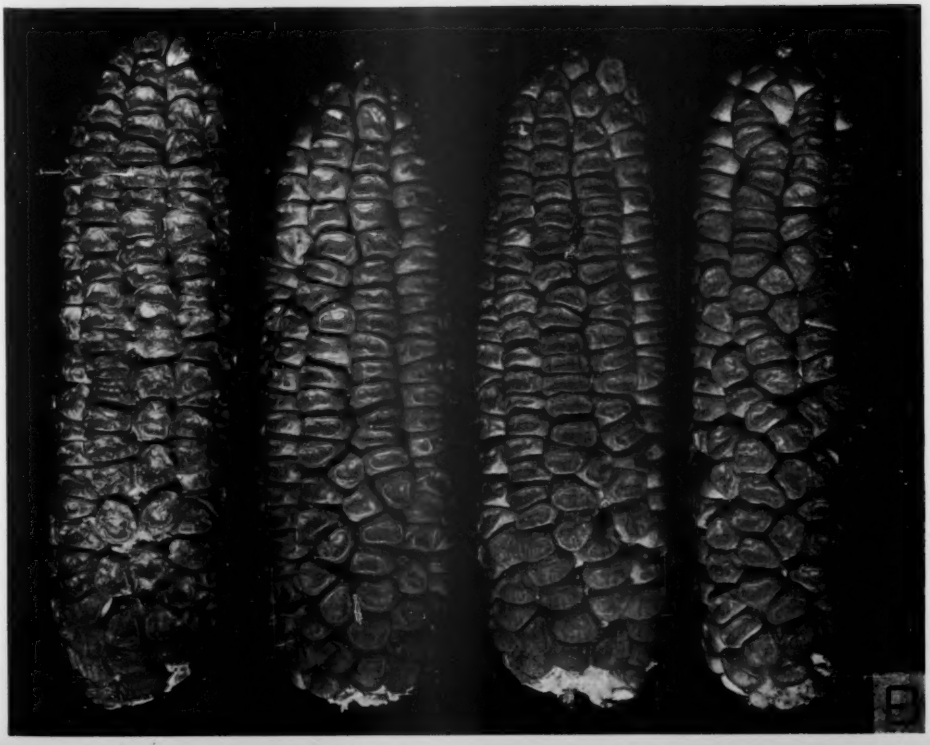
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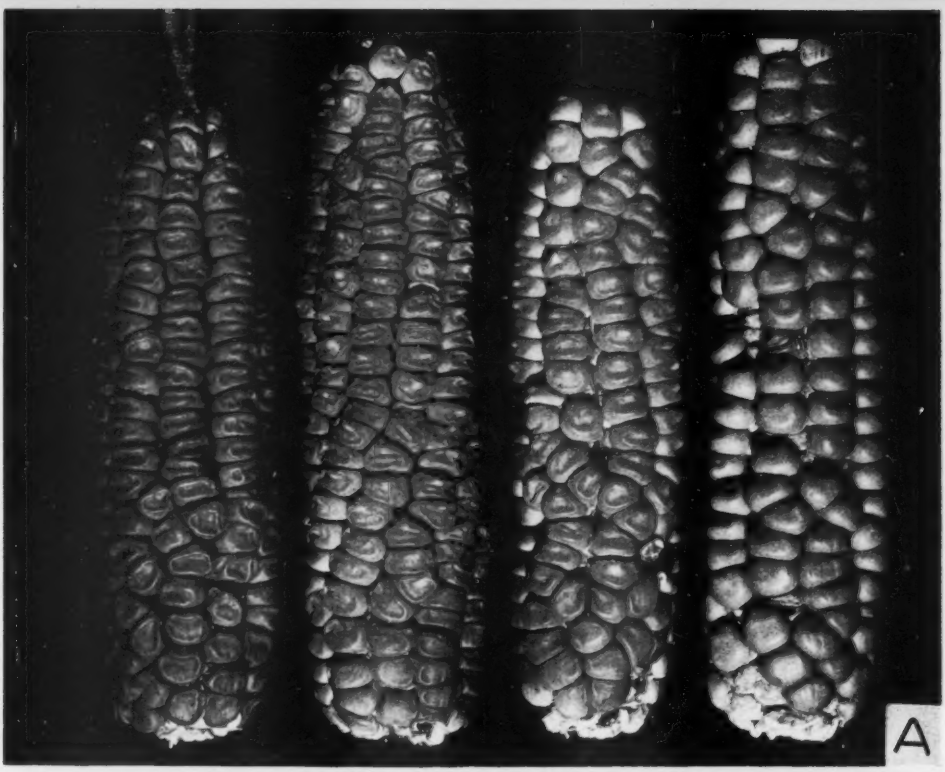
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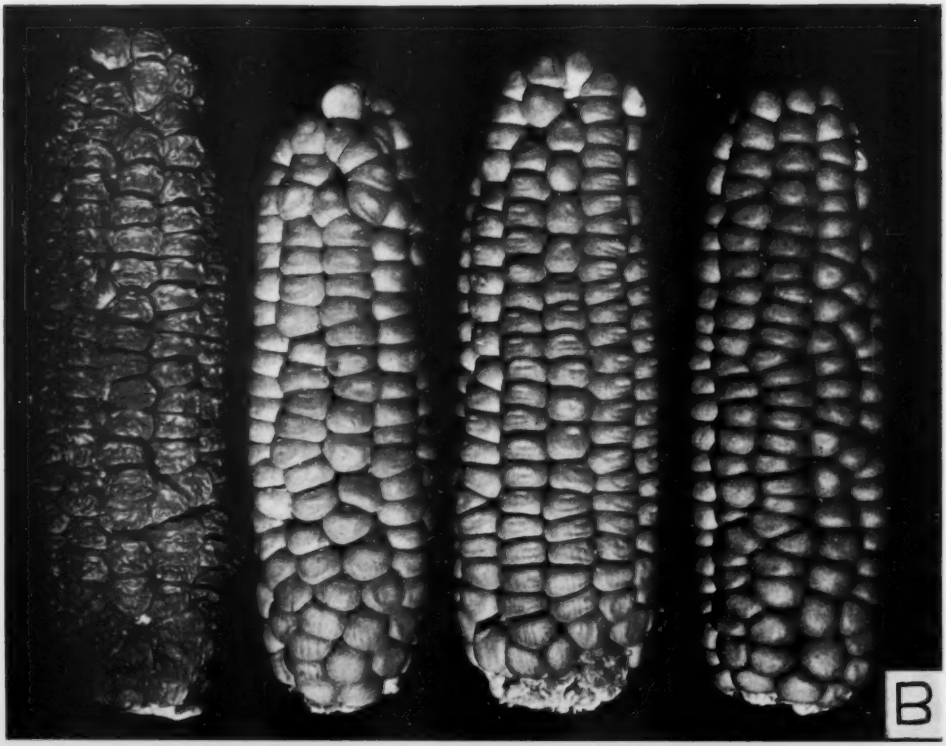
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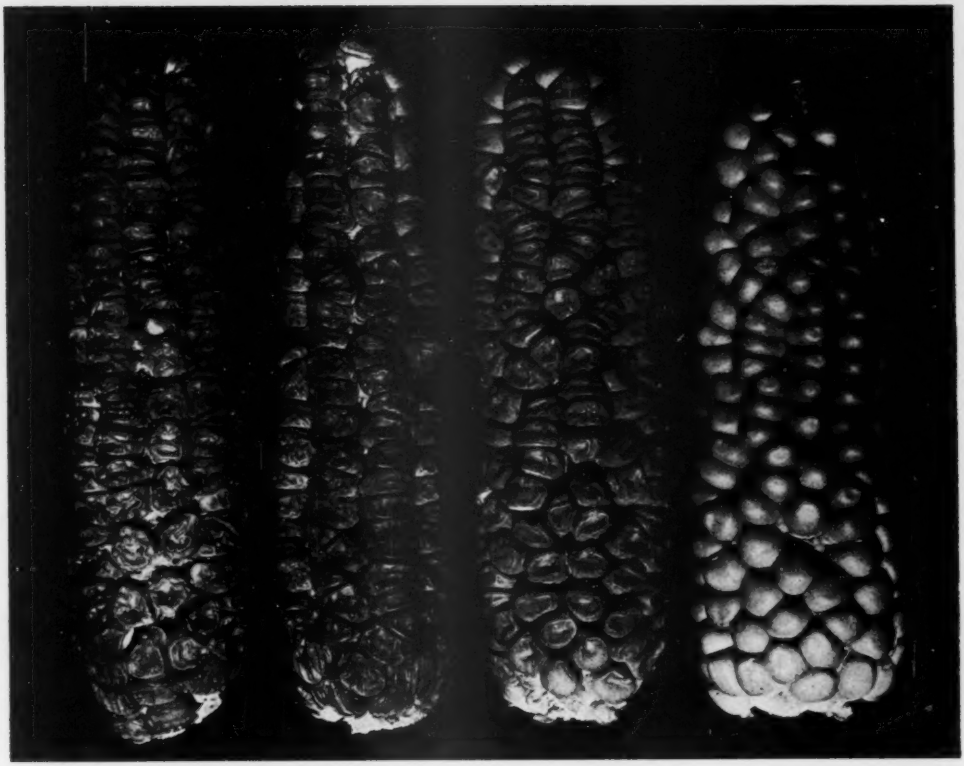

PLATE III

The four true-breeding endosperm types 0–0, 0–3, 3–0, and 3–3 (from left to right). These are respectively, supersugary, normal sugary, amylaceous sugary, and pseudostarchy. All except 3–3 are sugary-type.

PLATE II

FIGURE A.—The four endosperm genotypes 1–0, 1–1, 1–2, and 1–3 (from left to right). Genotype 1–0 is sugary-type, 1–1 is semistarchy (partly opaque) and the other two ears are starchy-type.

FIGURE B.—The four endosperm genotypes 0–1, 1–1, 2–1, and 3–1 (from left to right). These are superficially similar to the analogous dosage group in figure A. Genotype 1–1 appears in both figures. Two extremes were chosen to show the fluctuation possible.

Characteristics of the carbohydrate fractions

On the right hand side of table 1 are summarized the data on the various carbohydrate fractions measured, expressed as percents of the endosperm tissue on a dry weight basis. Reading upward from the bottom of the table, we see that reducing sugars, sucrose, and therefore total sugars decrease sharply with increasing total doses of the two higher alleles. Total water-soluble polysaccharides likewise decrease, while starch and total carbohydrates increase. The percent amylopectin also increases, with certain important exceptions. From a qualitative standpoint, this inverse relationship between sugars and water-soluble polysaccharides on the one hand, and starch on the other, substantiates results obtained by numerous other workers on endosperm types involving the gene pair, *su* and *Su*. To permit comparisons of the effects of increasing doses of either higher allele on backgrounds constant for the other, the data for starch and soluble polysaccharides have been regrouped.

The starch fraction

The starch data appear in table 2. Each of the 16 genotypes, with its respective percent of starch, appears once on each side of the table. In the left

TABLE 2

The percents of starch in the 16 endosperm genotypes in relation to increasing doses of Du, and to increasing doses of su^{am}, each on backgrounds constant for the other.

1	2	3	4	5	6	7	8
DOSAGE GROUP	DOSES PRESENT *su^{am} Du*	PERCENT STARCH	INCREASE† OVER NEXT LOWER GENOTYPE	DOSAGE GROUP	DOSES PRESENT *su^{am} Du*	PERCENT STARCH	INCREASE† OVER NEXT LOWER GENOTYPE
	3-3	80.7	3.0		3-3	80.7	4.7
4A	3-2	77.7	5.0	4B	2-3	76.0	6.3
	3-1	72.7	21.2		1-3	69.7	37.5
	3-0	51.5			0-3	32.2	
	2-3*	76.0	−3.6		3-2*	77.7	−1.9
3A	2-2	79.6	7.8	3B	2-2	79.6	9.3
	2-1	71.8	29.8		1-2	70.3	40.3
	2-0	42.0			0-2	30.0	
	1-3*	69.7	−0.6		3-1*	72.7	0.9
2A	1-2*	70.3	11.7	2B	2-1*	71.8	13.2
	1-1	58.6	25.0		1-1	58.6	31.4
	1-0	33.6			0-1	27.2	
	0-3*	32.2	2.2		3-0*	51.5	9.5
1A	0-2*	30.0	2.8	1B	2-0*	42.0	8.4
	0-1*	27.2	8.0		1-0*	33.6	14.4
	0-0	19.2			0-0	19.2	

* Identified for reference. See text.

† Expressed as additional parts of starch present, per hundred parts of tissue.

half, the material is presented in relation to increasing doses of *Du*, on the four possible backgrounds with respect to *su*^{am}. Four groups of four genotypes each are thus formed and these have been assigned the numbers, 1A, 2A, 3A, and 4A (column 1). In the right half of the table the genotypes are rearranged to show the effects of varying doses of *su*^{am} on backgrounds constant for *Du* (groups 1B, 2B, 3B, and 4B). Columns 4 and 8 show the increase in the percent starch brought about by any genotype as compared to the one next below it within its dosage group. For example, in group 1A genotype 0-0 produced 19.2 percent starch, while genotype 0-1 produced 27.2 percent. The difference, 8.0 percent, represents the additional number of parts of starch per hundred parts of tissue, which were produced by genotype 0-1.

Several conclusions may be drawn from these data. First, it is clear that the first dose of either gene was the most effective, on whatever background it was measured, while the second and third doses resulted in smaller increases. Thus in group 1A, the first dose of *Du* added 8.0 units of starch while the second and third doses added 2.8 and 2.2 units, respectively. In group 2A, the rise accompanying the first dose of *Du* was 25.0 units, followed by 11.7 and −0.6 units. The same type of response occurred in dosage groups 3A and 4A. The same effect is evident in the right half of table 2. In group 1B for example, the first dose of *su*^{am} produced 14.4 additional units and the second and third doses, 8.4 and 9.5 units. Here the succeeding doses appear to have produced more nearly equal effects than occur in other groups. Comment on this situation will be made later.

A second conclusion is that neither gene operating alone produces as much starch as do combinations of the two genes. This can be best seen in table 1, which shows that the seven sugary genotypes each produced less starch than any one of the starchy-types. Genotype 1-1 is particularly interesting in this relation. With but a single dose, each, of *su*^{am} and *Du*, its starch content was 58.6 percent, higher than that attained even by genotype 3-0 or 0-3. On the other hand, it was clearly lower in percent starch than any other genotype carrying both genes. This is in agreement with its usual semi-opaque and partially wrinkled appearance.

A third conclusion is that, dose for dose, *su*^{am} is more effective in starch production than is *Du*. Six independent pairs of genotypes containing equal total numbers of the two genes are available for comparison. They are 0-1, 1-0; 0-2, 2-0; 0-3, 3-0; 1-2, 2-1; 1-3, 3-1; and 2-3, 3-2. The members of these pairs appear in parallel positions on the left- and right-hand sides of table 2, and have been marked as a group for reference. In every case the percent starch produced by the right-hand member—the one containing the greater number of doses of *su*^{am}—was higher. The largest differences occur between members of dosage groups 1A and 1B. These provide a measure of the effectiveness of doses of either gene apart from the other.

The values in columns 4 and 8 indicate that *su*^{am} also maintained a higher *rate* of increase in starch production, per dose added, than did *Du*. From the common base line, genotype 0-0, the first dose of *su*^{am} (genotype 1-0) brought about a rise of 14.4 units, while that accompanying the first dose of *Du* was 8.0 units. The second dose of *su*^{am} added 8.4 units, and that of *Du*, 2.8 units.

The third doses added 9.5 and 2.2 units, respectively. The other independent series available for this comparison is composed of genotypes 2-1, 1-2, and 3-1, 1-3, from the common baseline, genotype 1-1. Here the differences in increase are small, but are in favor of *su*^{am}. The actual change in percent starch from genotype 1-2 to 1-3 was a decrease of 0.6 units. This and other such decreases are almost certainly due to fluctuations discussed under Methods.

Genotypes 2-3, 3-2, and 3-3, which are regularly opaque and smooth, were

TABLE 3

The percents of total water-soluble polysaccharides in the 16 endosperm genotypes in relation to increasing doses of Du, and to increasing doses of su^{am}*, each on backgrounds constant for the other.*

1	2	3	4	5	6	7	8
DOSAGE GROUP	DOSES PRESENT *su*^{am} *Du*	PERCENT TOTAL WATER-SOLUBLE POLYSACCHARIDES	DECREASE† FROM NEXT LOWER GENOTYPE	DOSAGE GROUP	DOSES PRESENT *su*^{am} *Du*	PERCENT TOTAL WATER-SOLUBLE POLYSACCHARIDES	DECREASE† FROM NEXT LOWER GENOTYPE
	3-3	0.4	0.0		3-3	0.4	0.6
4A	3-2	0.4	0.2	4B	2-3	1.0	0.8
	3-1	0.6	12.9		1-3	1.8	31.0
	3-0	13.5			0-3	32.8	
	2-3*	1.0	0.9		3-2*	0.4	1.5
3A	2-2	1.9	−0.2	3B	2-2	1.9	2.2
	2-1	1.7	16.1		1-2	4.1	28.1
	2-0	17.8			0-2	32.2	
	1-3*	1.8	2.3		3-1*	0.6	1.1
2A	1-2*	4.1	10.1	2B	2-1*	1.7	12.5
	1-1	14.2	11.7		1-1	14.2	21.7
	1-0	25.9			0-1	35.9	
	0-3*	32.8	−0.6		3-0*	13.5	4.3
1A	0-2*	32.2	3.7	1B	2-0*	17.8	8.1
	0-1*	35.9	4.9		1-0*	25.9	14.9
	0-0	40.8			0-0	40.8	

* Identified for reference. See text.

† Expressed as parts of water-soluble polysaccharides per hundred parts of tissue.

among the four highest in starch content. The fourth one, genotype 2-2, is that member of the intermediate group which is most often completely starchy in appearance.

Total water-soluble polysaccharides

The data on total water-soluble polysaccharides are regrouped in table 3. The arrangement is the same as that used for the starch data. Since the quantities of water-soluble polysaccharides grow smaller with added doses of *su*^{am} and *Du*, the changes tabulated in columns 4 and 8 are expressed as decreases. For

the sake of brevity, this fraction will be referred to as "soluble polysaccharides."

The behavior of this material shows patterns which are similar to, but in the opposite direction from those found for starch. The first doses of either gene were the most effective, this time in decreasing the percent of soluble polysaccharides. The effect is least marked in groups 1A and 2A, where the first dose of *Du* in each case resulted in only a slightly greater decrease than did the second dose.

The second observation complementing the data on starch is that *su*^{am} and *Du*, operating separately, did not cause decreases as great as those which occurred in genotypes carrying both higher alleles. The single exception was genotype 3-0, which in the material analyzed produced 13.5 percent of soluble polysaccharides. This is 0.7 units less than that found for genotype 1-1. All the other genotypes which contained both *su*^{am} and *Du* showed very low percents of this fraction.

The soluble polysaccharide data further agree with those on starch in showing that *su*^{am} was more effective than *Du*, dose for dose. Six independent pairs of genotypes are again marked (table 3) for identification. Within each pair the member which exceeded the other in doses of *su*^{am} produced the lower percent of soluble polysaccharides. The rate of change in this fraction, as was the case for starch, was usually higher for added doses of *su*^{am} than for those of *Du*.

Sugars

The data on reducing sugars and sucrose are presented in less detail. The amounts of these carbohydrates in mature endosperms are small, and it might be expected that such apparently residual substances would reveal little concerning the past action of the genes. The fact is, however, that the sugars for the most part show marked relationships to gene dosage. The data have not been reorganized into additional tables, but the values for any genotype can be found in table 1.

As compared to the soluble polysaccharides, certain differences are evident. Among genotypes 0-0, 0-1, 0-2, and 0-3 the percent reducing sugars was relatively high, but fluctuated widely (1.10 to 1.81 percent) and showed no relation to dosage of *Du*. One or more doses of *su*^{am} in the absence of *Du* (genotypes 1-0, 2-0, and 3-0) resulted in very much lower values (0.30 to 0.23 percent) but again not in order of the number of genes present. Finally, all nine of the genotypes carrying both *su*^{am} and *Du* showed very low percents of the fraction. Even among these latter the differential effect of *su*^{am} seems to be distinguishable. Thus genotype 1-1 had 0.14 percent reducing sugars; genotypes 1-2 and 1-3 showed but little change (0.14 and 0.11 percent); but genotypes 2-1 and 3-1, with added doses of *su*^{am}, produced only 0.06 and 0.05 percent, respectively.

In contrast to reducing sugars the behavior of sucrose followed quite closely that which has been described for the soluble polysaccharides. The absolute values were small, yet the same properties are reflected; namely, the first doses were the most effective, neither gene alone was as potent as the two together, and *su*^{am} was usually more effective than *Du*. The system departs from this pattern in one notable respect—doses of *Du* in genotypes 0-1, 0-2, and 0-3 did

not markedly decrease the percent sucrose from that found in genotype o-o. This is in agreement with the behavior of these same genotypes in regard to reducing sugars.

Amorphous, water-insoluble polysaccharides

The starch fraction sometimes included an amorphous, water-insoluble component. By the method used, the water-soluble polysaccharides were separated from the insoluble materials by centrifugation and under these conditions this component appeared as a surface layer in the precipitate. When wet it was gelatinous and opalescent; it gave a pale blue or lavender color reaction with iodine; and its presence could be correlated with the presence of the soluble polysaccharide fraction. Its occurrence as a surface layer—less readily precipitated than the other constituents—also suggests a relationship to the soluble fraction.

Only crude estimates of the quantity of the material were made, but relatively large amounts were present in sugary-type tissue while none could be identified in the most starchy types. When precipitates from equal starting weights of genotypes o-o, o-1, 1-o, and 1-1 were compared, genotype 1-1 showed a considerably smaller layer than did the others, and microscopic examination revealed this to be admixed with starch grains and cell wall particles. The corresponding layers from the three sugary types were fairly homogeneous and no difference in their quantities was evident. But in a similar comparison made between genotypes 2-o and o-2, the former definitely contained the larger quantity of this subfraction. The observation suggests that the higher values found for percent starch in genotypes 1-o, 2-o, and 3-o, as contrasted to o-1, o-2, and o-3, may in part represent greater amounts of this amorphous material. However, the whole of the differences in the proportions of soluble polysaccharides and starch among the sugary genotypes cannot be accounted for by this means. Observations made while isolating granular starch from other samples of the same genotypes indicated that marked changes in the quantities of starch grains also occur. Larger yields were obtained from genotypes 2-o and 3-o than from o-2 and o-3, and only small amounts could be recovered from genotypes o-o, o-1, and 1-o.

Total carbohydrates

It is apparent that a sensitive inverse relationship exists among the genotypes between the percent starch, on the one hand, and the percents of sugars and soluble polysaccharides, on the other. The carbohydrates show a further response to changes in genotype, which has long been known for *su* and *Su* kernels. Table 1 shows that the amounts of total carbohydrates were relatively low in the seven sugary types, and rose within the starchy class as additional doses of su^{am} and *Du* were added.

The data indicate three general groupings: Those lacking entirely either the gene su^{am} or *Du*, which contained from 60.4 to 67.6 percent carbohydrates; those comprising genotypes 1-1, 1-2, 1-3, 2-1, and 3-1, with from 71.7 to 74.7 percent; and the group 2-2, 2-3, 3-2, and 3-3, containing from 77.1 to 81.6 per-

cent. The position of genotype 2-2, with 81.6 percent carbohydrates, is probably abnormally high, since both 2-3 and 3-2 show lower values. The especially low values which occur in the two types 1-0 and 2-0, require comment. It was pointed out that these two did not show as high a rate of increase (over genotype 0-0) in percent starch as might be expected from the trends

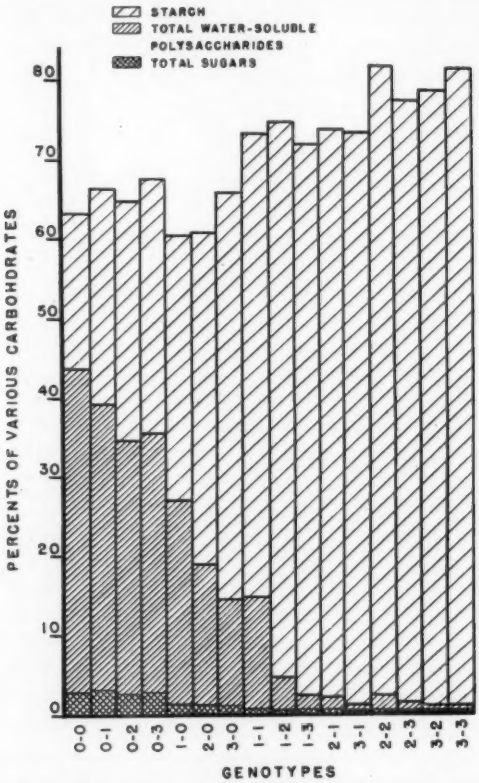

FIGURE 1.—The behavior of the system of 16 endosperm genotypes as regards quantities of starch, total water-soluble polysaccharides, and total sugars. Each column represents a genotype. Total heights of columns indicate the percents of total carbohydrates present.

within other dosage groups. The kernels available for analysis were in both cases somewhat less well developed and of a lower average weight than those of their companion genotype, 3-0. In a later crop (1946) which was not analyzed chemically, kernels of genotypes 1-0 and 2-0 were as plump and essentially as heavy as those of 3-0, which suggests that the causes for the low carbohydrate values were probably environmental.

The behavior of the system of carbohydrates has been portrayed by a histo-

gram (figure 1). The arrangement of the genotypes, from o-o to 3-3, is the same as that used in table 1. Three components—starch, total water-soluble polysaccharides, and total sugars—are indicated; the total height of each column represents total carbohydrates for that genotype. The trend from left to right toward higher totals is evident.

The forms of the starch grains

No extensive study of the differences in starch grain form has been made in the present work, but samples of the material isolated for amylose assay were examined microscopically. Such samples may not be completely random for the starch grain types occurring in the intact endosperm, since they were separated from treated tissue by centrifugation; nevertheless, the main characteristics for the various genotypes were clear.

The nine starchy types contained mostly simple grains showing considerable variation in size. No gross differences were evident from one genotype to another, except that compound grains appeared somewhat more frequently in genotype 1-1. Genotypes o-1, o-2, and o-3 showed typical compound grains interspersed with a few simple ones, and are therefore similar to other normal sugary types. Genotypes 1-o, 2-o, and 3-o, on the contrary, contained a definitely higher proportion of large, simple grains, which coincides with the higher percent of starch produced by the group.

In the case of genotype o-o, the appearance of the meager amounts of starch which could be recovered was different from that of any other sample. The grains were usually to be classed as compound, but the starch bodies were often poorly defined, and smaller and more numerous than in normal sugary genotypes. Some typical compound grains occurred, and even a few simple ones, but many others appeared misshapen, with rough, uneven surfaces. It is likely that these characteristics are related to the extremely high amylose content, described in the next section.

The amylose and amylopectin components of the granular starch

Granular starch from each genotype was analyzed to determine its content of amylose and amylopectin. Marked effects of the genes *su*^{am} and *Du* upon the ratio of these two starch fractions were found. The data, which appear in the right-hand column of table 1, are expressed as percent amylopectin. They are illustrated in figure 2 together with amylopectin values which have been found for certain other genetic types of maize and for a wrinkled variety of garden peas.

Within the 16 genotypes four classes may be distinguished. Genotype o-o stands alone with the lowest content of amylopectin, 35.0 percent. Genotypes o-1, o-2, and o-3, with 57.8, 59.6, and 61.4 percent amylopectin, respectively, form a second class whose members are set well apart from the pure-breeding supersugary genotype, but are very like one another. They show a small increase in amylopectin for each added dose of *Du*. A third class comprises genotypes 1-o, 2-o, and 3-o, with amylopectin values of 43.6, 48.9, and 50.0 percent.

Here, added doses of *su*^{am} increased the percent of amylopectin, with a spread in values greater than that found in the previous class. However these three genotypes, as a class, fall *between* the two groups already discussed. The remaining nine genotypes, each containing both *su*^{am} and *Du*, behaved in a rather surprising manner. Their amylopectin percentages, which were clearly higher than any of those among the sugary genotypes, were all essentially alike. The range is from 67.8 to 69.8 percent, with an average of 68.8 percent.

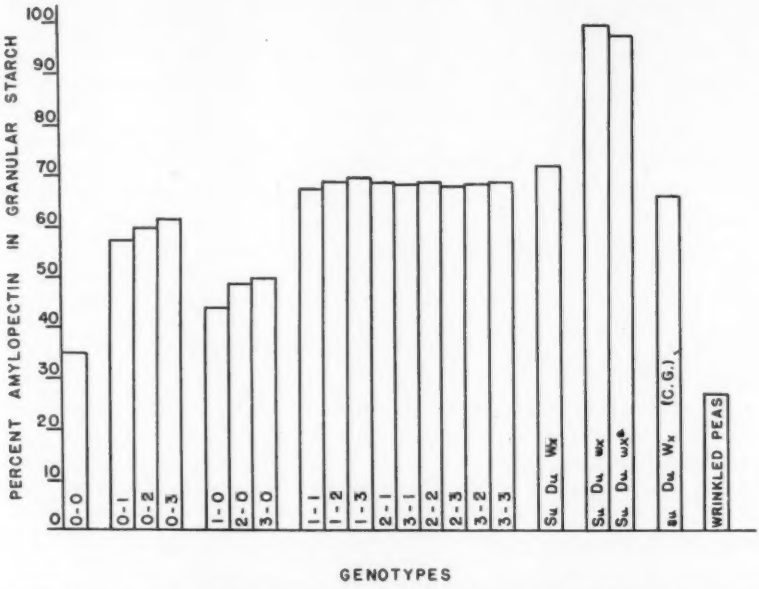

FIGURE 2.—The percent of amylopectin in the granular starch fractions of the 16 genotypes conditioned by *su*^{am}, *su*, *Du*, and *du*, and some other amylopectin values. *Su Du Wx* is ordinary corn starch; *Su Du wx* is waxy corn starch; *Du Su wx*^{a} is Argentine waxy; and *su Du Wx* is Country Gentleman sweet corn starch. The two waxy samples represent data of other workers.

The relationships among the genotypes, between the percent of granular starch which occurred as amylopectin and the amount of total starch laid down, are of considerable interest. In a scatter diagram (figure 3) each genotype is located with respect to these two properties. At the one extreme is genotype 0-0, with the least starch and the least amylopectin. At the other are the nine starchy-type members, closely grouped except for genotype 1-1, which is set apart by its lower starch content. Between these extremes the class constant for zero doses of *Du* is roughly intermediate both in respect to total starch and to the amylopectin component. However, the analogous class which lacks the gene *su*^{am} is at once high in amylopectin and low in total starch.

There is thus definite correlation between the two properties, but the rela-

tionship is not linear. An interpretation is suggested later in the paper. Comment is pertinent here on the other amylopectin values shown in figure 2. The column marked *Su Du Wx* has a value of 72.3 percent, and represents common starchy maize, homozygous for each of the three genes named. This is the type of starch reported as "*Wx Wx Wx*" by BRIMHALL, SPRAGUE, and SASS (1945); a sample of their preparation was analyzed as a control in the present series.

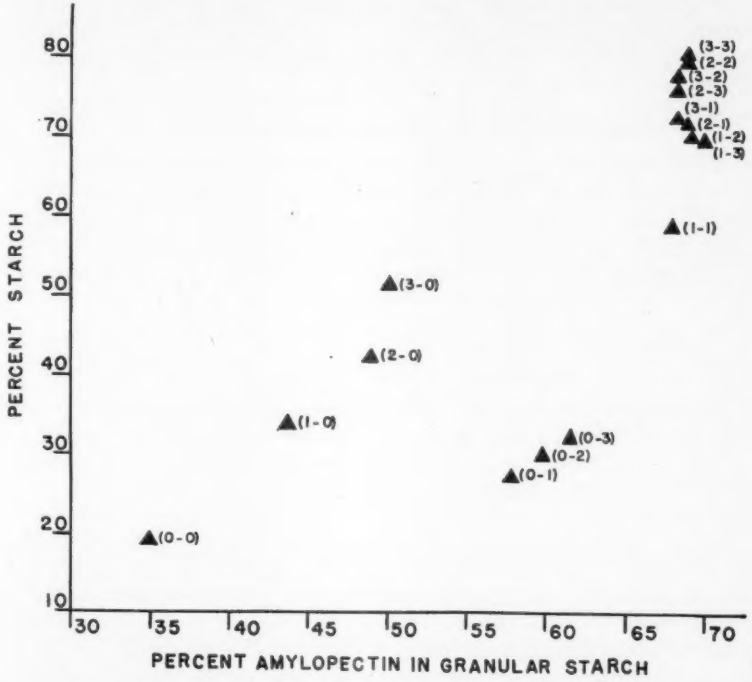

FIGURE 3.—The relationships between percent total starch and percent of the granular starch which occurred as amylopectin in the 16 endosperm genotypes. The members fall into four principal groups on the basis of genotype.

Considering the accuracy of the analytical method, the percent amylopectin is probably significantly higher than those found for the starchy types, 1-1, 1-2, 1-3, *etc.* The latter are genetically different in that they do not contain the allele *Su*, but since two different strains of maize are involved, the difference in amylopectin could be due to modifying factors.

A sample of Country Gentleman sweet corn starch was found to contain 66.0 percent amylopectin. It is identified in figure 2 as *su Du Wx* (C.G.). Since it is identical to the normal sugary genotype, 0-3, as regards the genes named, it seems probable that here the different modifying complexes are affecting the

percent amylopectin. Two other genetic types analyzed by BRIMHALL, SPRAGUE, and SASS (1945) are illustrated. They are waxy maize (*Su Du wx*) with 100 percent amylopectin, and Argentine waxy (*Su Du wx*^{a}) with 97.6 percent. These display proportions of branched-chain starch which are quite different from those which have been discussed. Despite this change, the total starch content of waxy maize is essentially as high as that of other non-sugary types.

The starch from wrinkled (sugary-type) garden peas contains a very low percent of amylopectin (NIELSEN and GLEASON, 1945; HILBERT and MACMASTERS, 1946). The writer assayed a sample prepared by DR. BRIMHALL and obtained a value of 27.1 percent.

Subfractionation of the total water-soluble polysaccharides

Certain endosperm types were analyzed for their content of two subfractions, called glycoamylose and phytoglycogen (SUMNER and SOMERS, 1944). In one experiment the four pure-breeding genotypes were compared. The total water-soluble fraction was first obtained and the yields, expressed as percent of the air-dry, whole kernels, were as follows:

pseudostarchy	(3-3)	0.7
amylaceous sugary	(3-0)	8.3
normal sugary	(0-3)	25.0
supersugary	(0-0)	38.0

They are in good agreement with those presented in table 1 for the same genotypes, considering the differences in the manner of calculation.

The results of subfractionation of this material, and of that obtained from another maize cross, are shown in table 4. The four pure-breeding genotypes have been arranged in order of increasing amylopectin content. Above them are shown two endosperm types which arose from a cross of the starchy inbred line, Minnesota A158 (*Su Du Wx*) with a genetic stock (*su Du wx*). By a backcross of this F_1 by its waxy parent, waxy and non-waxy kernels were obtained upon the same ear. The amylopectin content of these two samples was not measured, but a value of 100 per cent is expected for the waxy maize; the non-waxy sample being of the genotype 0-3 as regards *su* and *Du*, should contain a percentage similar to that of other normal sugary types.

The distribution of the two subfractions shows a marked relationship to the amylopectin percentages. The recoveries represent about ninety percent of the starting material, as explained in Methods. From genotype 0-0, phytoglycogen totaled 39 parts, with 49 parts recovered as glycoamylose. In genotype 3-0, with a higher percent of amylopectin, the proportion of phytoglycogen increased, and in genotype 0-3 it showed a further rise, the ratio being 66:27. Genotype 3-3 did not yield sufficient "solubles" to permit a subfractionation, but it has been included to complete the series for amylopectin. The normal sugary genotype from the waxy cross is in good agreement with its counter-type, 0-3, having 60 parts of phytoglycogen and 29 parts of glycoamylose. Its companion type, waxy, showed a unique behavior. Of the 94 parts recovered, 93 separated as phytoglycogen, leaving only 1 as glycoamylose.

The relative ease with which phytoglycogen could be separated from glycoamylose, among the various genotypes, is also to be noted. In the waxy maize (table 4) 92 of the 93 parts were recovered in the first separation. For the non-waxy companion sample the proportion was 52 parts out of 60, a figure which was also closely approximated by genotype o-3. For amylaceous sugary there was a further decrease in the proportion, and finally, in genotype o-o, only 8 parts out of a total of 39 were recovered in this first separation.

The color reactions of one to two percent solutions of these polysaccharides with a weak solution of iodine in potassium iodide were examined. Macroscopi-

TABLE 4

The distribution of two subfractions of the total water-soluble polysaccharides in various endosperm genotypes, and their relationships to the percent of amylopectin.

SOURCE OF MATERIAL	HOMOZYGOUS ENDOSPERM GENOTYPE	AMYLOPECTIN IN GRANULAR STARCH (FROM TABLE 1)	DISTRIBUTION OF WATER-SOLUBLE POLYSACCHARIDES					
			PHYTOGLYCOGEN				GLYCOAMYLOSE	
			1ST SEPARATION %	2ND SEPARATION %	TOTAL %	I_2 COLOR	%	I_2 COLOR
Minn. A 158×waxy, F_1	*su Du wx*	(100)†	92	1	93	brown	1	red-purple
waxy	*su Du Wx**	(±65)†	52	8	60	brown	29	blue
Pure-breeding genotypes of table 1	(3-3) su^{am} *Du Wx*	68.7	None available from this genotype					
	(o-3) *su Du Wx*	61.4	58	8	66	brown	27	blue
	(3-o) su^{am} *du Wx*	50.0	30	19	49	brown	42	blue
	(o-o) *su du Wx*	35.0	8	31	39	brown	49	deep blue

* This endosperm genotype was *Wx Wx wx*.

† Expected values. These samples were not analyzed for percent amylopectin.

cally all subfractions classed as phytoglycogen, either by the first or the second separation, stained brown. All glycoamylose fractions (with more variation) stained blue. This substantiates the report of SUMNER and SOMERS (1944), who cited these color reactions among the criteria for identification of the two subfractions. Microscopic examination revealed, however, that both blue- and brown-staining aggregates existed within the glycoamylose fraction, although these were composed of grossly similar, amorphous material. Similar aggregates in the phytoglycogen fractions were almost always brown under the microscope. Genotype o-o yielded glycoamylose which stained a deeper blue than any other. This is also the genotype which contained the highest percent of amylose. The small quantity of material which separated as glycoamylose in the waxy sample on the contrary, stained red-purple, which indicates the presence of fairly short glucose chains.

The whole behavior of this system points to a connection between amylopectin and phytoglycogen on the one hand, and amylose and glycoamylose on the other. The names assigned to the soluble fractions by SUMNER and SOMERS

suggest that these authors had such relationships in mind, but they drew no analogies.

It should not be inferred from the evidence presented that glycoamylose and phytoglycogen represent well-defined molecular species, nor that the numerical values shown for them are an exact measure of their proportions. The brown-staining aggregates seen in the glycoamylose fractions show that the latter are not "pure." It can be assumed, however, that in phytoglycogen long unbranched chains which would cause a blue color are absent.

An attempt was made to separate the two subfractions from samples of total soluble polysaccharides which had been obtained from the residues from sugar extractions. The proportions which were recovered as glycoamylose from a sample of genotype 0-3 were greater than those shown in table 4, and duplicate fractionations did not agree. Samples of waxy maize were then treated in the same manner, and again larger proportions separated as glycoamylose than had previously done so. It is not considered that these results invalidate the conclusions drawn from table 4. They do indicate the sensitivity of such a system of glucose polymers to experimental treatment.

The breakdown of glucose-1-phosphate in the presence of extracts of developing kernels

An experiment was carried out with extracts of developing kernels in an effort to detect any differences in what was presumed to be phosphorylase activity (BLISS and NAYLOR, 1946; HANES, 1940a). The breakdown of glucose-1-phosphate by the enzyme extracts was measured by the increase in inorganic phosphate upon incubation of a test solution. Colorimetric phosphorus determinations were made after 5, 15, and 45 minutes incubation. Extracts from kernels taken 22 days and 32 days after pollination were studied. Inorganic phosphate was liberated by all of the extracts, although at a low rate. The genotypes measured at the 22 day stage were 0-0, 0-1, 1-0, 1-1, 0-3, 3-0, and 3-3. The two starchy types, 1-1 and 3-3, showed a slightly higher rate of liberation of phosphate than the sugary ones.

At the 32 day stage five genotypes, 0-0, 0-3, 3-0, 1-1 and 3-3 were assayed. The sugary members of this series (and the starchy member, 3-3, when assayed in high concentration) displayed an interesting phenomenon detected primarily because of the method being used. The bulk of the glucose-1-phosphate added to the test solutions did not yield free phosphate during incubation, but being acid-labile it continued to break down in the acid system used for the colorimetric determinations. This action was taken into account, since the readings were always made on a fixed time schedule. It was found, however, that for the genotypes cited the mixtures which had been incubated for 15 and especially for 45 minutes yielded additional phosphate under the influence of the acid at a markedly higher rate than did the five minute samples, suggesting that a compound more acid-labile than glucose-1-phosphate had been formed. One such compound, acetyl phosphate, has recently been reported by LIPMANN (1940).

DISCUSSION

Gene dosage effects on the endosperm carbohydrates

The quantitative effects of the genes *su*^{am} and *Du* are clearly reflected in the data which have been reported. These genes show incomplete dominance, both individually and in their combined action. The first doses are, however, more effective in most respects than are additional ones. Strictly speaking, the effects of any genotype save 3-3 are conditioned also by the doses of *su* and *du* present but it is unlikely that this influence is great. The fact that the addition of the higher alleles results in more of some components and less of others need not be a cause for concern. As discussed later, the separate fractions probably all belong to an integrated system for polysaccharide synthesis.

A similar situation where dominance is incomplete but of a high degree has been shown in maize for the gene *Wx*, as regards its effect on amylose production (SPRAGUE *et al.*, 1943). The relative amounts of the straight-chain starch produced in the presence of zero, one, two, and three doses of *Wx* were represented by the series 0.0, 18.4, 20.4, and 22.0.

The gene *Su*, being an allele of *su* and *su*^{am} is of particular interest. The data of LINDSTROM and GERHARDT (1926, 1927) showed that as regards the ratio of total sugars and dextrins to starch, the dominance of *Su* over *su* is practically complete. Furthermore (MANGELSDORF, 1947), the appearance of *Su* kernels is only slightly affected by *du* in the homozygous state, there being an occasional slight wrinkling and dullness. The amylopectin content, too, seems to be but little affected. BRIMHALL and SPRAGUE (unpublished) analyzed kernels of the genotype *Su ? ? du du du;* these showed 69 percent amylopectin, which is only slightly different from that of other starchy kernel types.

The gene *Su*, however, does show incomplete dominance in crosses with *su*$_x$ (HOROVITZ, MARCHIONI, and FISHER, 1941). The endosperm genotypes reported are analogous in many respects to those of the present series. The allele *su*$_1$ is concerned in both cases. In Horovitz' *et al.* series, *Su*$_1$ supplants *su*$_1^{am}$ while *Su*$_x$*su*$_x$ take the place of *Du du*. Kernels of the constitution *Su*$_1$ *? su*$_1$ *su*$_x$*su*$_x$*su*$_x$ were typically sugary in appearance, which indicates that *su*$_x$ is more deleterious to the production of starch than is *du*. These kernels also had a higher sugar content than *Su*$_1$*Su*$_1$*Su*$_1$ *su*$_x$*su*$_x$*su*$_x$ kernels. Combined samples of the genotypes *Su*$_1$*su*$_1$*su*$_1$ *su*$_x$*su*$_x$*su*$_x$ and *su*$_1$*su*$_1$*su*$_1$ *su*$_x$*su*$_x$*su*$_x$, were reported to contain 13.8 percent of total sugars, which to the writer's knowledge is the highest value which has been found in mature maize seed. SHAFER (1946) after crossing *su*$_x$ with the *su*$_1$ inbred, P51, also recovered in the F_2 a supersugary class.

Relationships between the soluble and insoluble carbohydrates

The interpretation of the relationships between the various carbohydrate fractions is important to any explanation of the heritable mechanisms involved. It is considered here that the sugars in mature kernels are primarily residual, that the soluble and amorphous insoluble polysaccharides represent intermediates in a starch-synthesizing system, and that the quantities of these

components reflect the degree of completion to which the reactions have proceeded. The close correlations which have been shown for the changes in these quantities from genotype to genotype are in accord with this concept. Abundant evidence (see Introduction) shows that for starchy and sugary types in general, the carbohydrates present during development can be satisfactorily interpreted in the same manner.

The evidence on sugars is worthy of note. BERNSTEIN (1943) found that early in development, sugars formed 20 to 30 percent of the dry weight of the endosperm. Reducing sugars were highest about one week after pollination and then decreased in percentage, while sucrose did not reach its peak until the second or third week, when starch development was beginning. There was a high statistical correlation between sucrose content and rate of starch production throughout development, suggesting that this sugar serves as a source material for starch synthesis. It has been shown that sucrose is probably the most important form in which carbohydrate is transported in the maize plant and that its movement is polarized toward the ear during fruiting (LOOMIS, 1945). In the present data, it is residual sucrose rather than reducing sugars, which shows a close inverse relationship to starch content. This may indicate a key role for this compound in polysaccharide synthesis.

It has sometimes been considered, especially before the elucidation of the phosphorylase enzyme system, that the accumulation of the water-soluble polysaccharides might be due to starch-hydrolyzing amylases. PARKER (1935) attacked this problem indirectly, using sweet corn at two stages during development as well as at maturity. He separated the soluble carbohydrates and the starch each into two parts by electrodialysis, and compared several properties of the corresponding fractions. PARKER's starch fractions were probably degraded mixtures of amylose and amylopectin (as now defined), and the relationships of the two intermediate fractions to phytoglycogen and glycoamylose cannot be stated. He concluded, however, that as the corn matured the properties of the water-soluble fractions approached those of their proposed counter-fractions of starch and did not agree with the properties of artificially produced breakdown products. Earlier LAMPE (1931) had found no morphological evidence of hydrolytic action in developing sugary types, although the accumulation of globules of liquid carbohydrate was a prominent feature.

BERNSTEIN (1943) investigated the possibility that amylases might be acting reversibly in the synthesis of polysaccharides. Using developing maize endosperm he measured *in vitro* the amylase activities of extracts of starchy, waxy, and sugary types. No α-amylase was detected and the activity of β-amylase was low in all samples. There was no positive correlation between β-amylase and the rate of starch production. BERNSTEIN concluded that there was little evidence for a synthetic function of these enzymes, and noted that the phosphorylase system was more likely responsible. It is significant to the present discussion that his sugary maize appears to have had no more amylase activity than any of the other types. There is thus little indication from any of these

investigations for origin of the soluble polysaccharides by hydrolysis of a part of the starch.

Correlations between genic effects and the biochemistry of polysaccharide synthesis

Since enzymes of the phosphorylase system are fundamental to polysaccharide synthesis, it is reasonable to suppose that they are concerned with the effects described in this paper. Among the carbohydrate changes, the most striking and in the writer's opinion the most significant is that in the proportions of the straight and branched-chain molecules which compose the starch grains. This change points to gene control over the activities of the enzymes responsible for formation of these molecules. (Such control had already been indicated in the case of waxy.) Many of the other carbohydrate changes may be dependent upon these altered activities.

Any attempt to become more specific concerning the nature of the genetic effects might seem unwise, considering the complexities surrounding carbohydrate metabolism. Polysaccharide synthesis represents a part of an involved system of reversible interrelated reactions having to do with energy release (see MEYERHOF, 1941). Furthermore, the specific conditions which affect the action of phosphorylase are several. GREEN and STUMPF (1942) showed that the rate of formation of polysaccharide *in vitro* by purified phosphorylase varied with the concentration of the enzyme, of the substrate, and of the starch present as catalyst, as well as with the pH and the temperature. Some of the characteristics of "Q" enzyme, which appears to form the linkages resulting in branch points in the starch molecule, have been described by BOURNE and PEAT (1945)and BOURNE, MACEY, and PEAT (1945)

It is the properties of the two enzymes, however, which are most suggestive in regard to effects observed among the 16 genotypes. (In what follows individual references will not always be quoted; the material may be found in the comprehensive papers by CORI, SWANSON, and CORI (1945) and HASSID (1945) and in those papers cited in the previous paragraph. The experiments are not to be credited to the writer.) The action of P enzyme (phosphorylase) consists in lengthening a polyglucose chain by the successive addition at the non-reducing end, of units derived from glucose-1-phosphate. Its action alone would result in long, unbranched polymers of the nature of amylose. Purified P enzyme, however, seems not to *originate* glucose chains; therefore the rate at which it can synthesize additional polysaccharide is limited, at least *in vitro*, by the number of non-reducing ends of terminal glucose units present. The absolute length of the polymers carrying these end groups also affects the activity of P. In experiments where amylose was used, little additional synthesis by purified muscle phosphorylase has been reported even though the total number of end groups present was high.

When enzyme preparations containing both P and Q are employed, higher rates of synthesis and polymers of the amylopectin type result. This has been interpreted as being due to the initiation of branch points by Q enzyme fol-

lowed by lengthening of these new side chains by P. (The average length of the branches in amylopectin is about 20 to 25 glucose units; BOURNE and PEAT (1945) have postulated that it is entire chains of such a length which Q enzyme links together.) It has been pointed out by BATES, FRENCH, and RUNDLE (1943) that there is no extensive intergradation in structure between amylose and amylopectin although they occur together in common types of starch. These authors conclude that Q enzyme does not act at random, for the total number of branch points which can be determined in whole starch from non-sugary varieties of corn or from potato is such that were they randomly distributed, only about one percent of material with the properties of amylose would occur.

The carbohydrate data in the present paper suggest that changes in the functioning of these enzyme systems are conditioned by the gene changes. If less Q enzyme were available in a particular genotype the ratio of amylose to amylopectin formed should rise. However, this involves there being fewer branch points and thus fewer added end groups for P to build upon. Since P appears not to be able to lengthen a chain indefinitely, the entire process of polysaccharide accumulation would be impaired. This occurs in sugary endosperm types.

In the present genetic system each gene pair, separately, affects the starch content and the amylose: amylopectin ratio but the amount of change produced by the two genes and for the two properties is different. Genotypes 0-1, 0-2, and 0-3 contain nearly as high a proportion of amylopectin as the starchy types, yet they show less total starch than 1-0, 2-0, and 3-0 which are considerably lower in amylopectin. Something more than a single effect on the availability of Q enzyme is indicated. Although a specific action cannot be assigned with certainty to either gene, the situation might be explained by postulating an action of *Du* on Q enzyme and of *su*^{am} on the origination of short glucose chains. If in genotype 0-0 both were limited, with P enzyme present in excess, a relatively small amount of total polysaccharide of which a high proportion would be straight-chain, should result. A dose of *Du* (as in genotype 0-1) would increase the available Q enzyme with a resulting increase in the percent amylopectin. A dose of *su*^{am} (genotype 1-0) by increasing the availability of original short glucose chains should increase the rate of synthesis brought about by P. To account here for the rise in the proportion of amylopectin, the adaptation of P to short chains and the non-random nature of the action of Q in forming branch points could be accepted. That is, P enzyme having additional short chains to which to add glucose units would less often lengthen a chain beyond the point where Q could form a branch. Additional branches and therefore additional material of the nature of amylopectin might thus be formed without any absolute increase in Q enzyme. The sharp rise in starch, amylopectin, and total carbohydrates which accompanies the presence of both genes together would reflect the higher efficiency of the system when the respective limiting materials were both supplied in greater abundance. Additional doses of these materials would sooner or later be expected to result in diminishing effects.

Regardless of the accuracy of such an hypothesis, the association of high amylose content with low total starch, compound starch grains, and the accumulation of much of the polysaccharide in unorganized form is probably significant. These same conditions also occur together in garden peas as the result of a single recessive gene. However, sugary, waxy maize with 100 percent amylopectin also shows these characteristics. Differences in the rate of synthesis of total polysaccharides as well as in the adaptability of amylose and amylopectin to starch grain formation may be involved.

The data on this carbohydrate system are interpreted to indicate that genes governing quantitative inheritance may not condition reactions any more complex than those accompanying simple qualitative changes. The genetic effects in the present case are probably not the result of "all or none" reactions, but rather of changes in the degree to which processes proceed which occur even in the lowest member of the series. The several changes in end products can still be attributed to simple, specific changes in the action of the genes themselves.

SUMMARY

1. The effects of the 16 possible combinations of two gene pairs, $su_1^{am}su_1$ and *Du du*, on the amounts and kinds of carbohydrates in mature maize endosperm were measured.

2. The seven genotypes lacking su_1^{am}, or *Du*, or both, were phenotypically sugary-type while the nine which carried one or more doses of both genes were starchy-type. Gradations in appearance occurred within the two groups.

3. The percent of starch increased, and that of sugars and water-soluble polysaccharides decreased in a definite pattern with increasing doses of su_1^{am} and *Du*. For each component the first dose of either gene was the most effective; a dose of both genes was more effective even than three doses of either alone; and su_1^{am} was more effective than *Du*.

4. The behavior of sucrose was more clearly correlated with that of the polysaccharides than was the behavior of reducing sugars.

5. An amorphous water-insoluble polysaccharide was observed and is considered to be material intergrading between soluble polysaccharides and starch.

6. The proportions of amylose and amylopectin in the granular starch were determined. Amylopectin was lowest (35 percent) in the most sugary genotype and highest (about 69 percent) in the starchy ones. Among certain intermediate genotypes the relationship was reversed.

7. Two subfractions of the water-soluble polysaccharides, known as glycoamylose and phytoglycogen, were examined in certain genotypes. The proportions of these subfractions varied essentially as did those of amylose and amylopectin. Their respective iodine colors were also similar.

8. Extracts of developing kernels could be shown to cause the breakdown of glucose-1-phosphate. Starchy types showed slightly higher rates than sugary ones.

9. The carbohydrate fractions studied are considered to be closely interrelated and to reflect the polysaccharide-synthesizing activities of the genotypes. Possible roles of enzymes of the phosphorylase system are discussed.

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THE INDUCTION OF INHERITABLE MODIFICATION IN REACTION TO ANTISERUM IN PARAMECIUM AURELIA[1]

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ANTISERUM produced by injecting *Paramecium aurelia* into rabbits ordinarily immobilizes and frequently kills paramecia of the stock used for injection. Several workers have been able to obtain from such sensitive stocks paramecia resistant to the action of the antiserum, and the resistance was transmitted to the descendants for varying periods. This work has recently been reviewed by SONNEBORN (1947), but some of the more important features must be mentioned here.

JOLLOS (1921) reported that he was able to produce resistant animals which transmitted the resistance to their progeny by fission from a few weeks up to two months before it finally disappeared. This was one of the cases upon which he based his concept of "Dauermodifikationen" or long-lasting modifications. His technique was to expose animals of a sensitive clone to antiserum, select the few most resistant animals, culture them in the absence of antiserum, expose the descendants to a higher concentration of antiserum, and repeat the selection process. By such repeated selections, he was able to obtain lines which were highly resistant to antiserum. It is interesting in view of what is to be reported here that he obtained one highly resistant line by a single selection. He believed that the resistance was due to a modification of the cytoplasm and not of nuclear components since it was of transitory nature and disappeared during long continued vegetative multiplication or after one fertilization.

SONNEBORN (1943 a, b) working with stock 60 of variety 1 of *Paramacium aurelia* also obtained apparent "Dauermodifikationen" in reaction to antiserum. About 20 percent of the animals of certain clones of this stock were normally resistant to homologous antiserum, but this resistance was lost in a very few fissions. However, when such resistant animals were selected from a serum test and samples of their progeny were tested from time to time to see if they were resistant, they demonstrated that resistance persisted for periods up to 279 fissions before finally disappearing. Thus a single exposure to antiserum appeared to transform a very transient resistance into a long-lasting one. SONNEBORN pointed out the similarity to JOLLOS' work and concluded as JOLLOS that the changes could not be due to "an ordinary persistent mutation."

HARRISON and FOWLER (1945) carried out an extensive study of the effect of selection upon reactions to antisera in *P. aurelia*. By selection of resistant

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and sensitive animals as soon as they could be recognized (less than two hours in antiserum), they established both more resistant and more sensitive lines from their original stocks. Some of the variant lines reverted to normal in three to fourteen months while others persisted for the full seventeen months of the experiment. The results are extremely varied and, as SONNEBORN (1947) has pointed out, quite possibly include several different phenomena. The authors apparently interpret their results as due to selection of naturally occurring variants and not to an inducing action of the antiserum. Since the opposite view will be developed in this paper, it should be pointed out that they chose for study stocks in which much variation in serum reaction did occur and exposed the animals for very short periods to antiserum. In the present study, a very stable stock has been used and long exposures to antiserum employed.

These studies raise a number of questions. Does the antiserum act by selecting naturally occurring variants as HARRISON and FOWLER suggested, or does it induce the changes as JOLLOS believed? What part of the genetic mechanism is affected? Is it the cytoplasm as JOLLOS believed, the micronucleus, or the macronucleus as SONNEBORN (1947) has suggested on p. 314 of his review?

As SONNEBORN (1947) has pointed out, the recent work upon the self-duplicating cytoplasmic factor kappa suggests a possible cytoplasmic mechanism for such changes. SONNEBORN (1947) and PREER (1946) have shown that non-killer lines can be produced from killer clones by rapid multiplication or by exposure to high temperature. Some of these lines reverted to killer after some fissions while others remained permanently non-killer. The changes have been shown to be due to a reduction in the amount of kappa present, the temporary non-killers resulting from a great reduction but not a complete loss of kappa, the permanent non-killers resulting from the complete loss of kappa. The similarity of these phenomena to some of the cases of "Dauermodifikationen" is striking.

The presence of cytoplasmic factors such as kappa has up to now been demonstrated only for varieties belonging to the B group in *P. aurelia*. The main evidence for their existence has come from the observation that the clones derived from the two members of a conjugant pair differ from one another in accordence with the origin of their cytoplasm. Such factors have not been demonstrated in the A group of varieties to which variety 1 belongs. If "Dauermodifikationen" in variety 1 can be shown to be due to a mechanism similar to that involved in the production of temporary non-killer lines, a greater degree of uniformity will have been brought into our picture of the genetic mechanism of *P. aurelia* and cytoplasmic factors will be shown not to be just a peculiar feature of the B group of varieties. A preliminary report indicating that this is so was made by KIMBALL (1947).

MATERIALS AND METHODS

Stock 60 of variety 1 of *P. aurelia* was selected for this study. This is the same stock used by SONNEBORN (1943 a, b) in his studies of antiserum variations. The stock was kindly supplied by Professor SONNEBORN, whose interest and stimulating discussion have been of the greatest value in this work.

Antiserum against this stock was prepared according to method #4 of McClung (1944). The antiserum was heated to 60°–65°C for 30 minutes before using and then diluted to the desired concentration with a modification of the salt solution described by Bernheimer and Harrison (1940): $NaHCO_3$—0.02 g; $MgCO_3$—0.03 g; $MgCl_2$—0.016 g; $CaSO_4$—0.071 g; $CaCl_2$—0.001 g; glass distilled water—1000 cc.

EFFECT OF ANTISERUM ON UNTREATED PARAMECIA

The paramecia were tested for reaction to antiserum by placing one to fifteen animals in a drop of diluted antiserum—one part antiserum to 39 parts salt solution in most of the work; one part antiserum to 79 parts salt solution in some later work. In either of these concentrations, animals of stock 60 were uniformly immobilized within two hours and killed within twenty-four hours. The exceptions to this were very rare indeed. Occasionally animals which were still twitching slightly were found after two hours. Still more rarely animals were found which were able to move about very slowly. Only one clear case of spontaneous resistance was found. This case involved several different animals taken from a single culture. These statements are based upon observations of thousands of animals of many different clones of stock 60.

Sonneborn (1943, 1947) reported that certain clones of stock 60 showed about 20 percent spontaneous resistance to antiserum. It is possible that the one exceptional case mentioned above was a clone of this sort, but such clones were certainly exceedingly rare in the present work if they occurred at all.

The constancy of the reaction made it safe to classify as "resistant" any animals which moved slowly about from place to place after two hours exposure to 1/40 or 1/80 antiserum. Actually a considerable portion of the animals so classified moved about quite rapidly or showed no retardation of movement at all. A distinction between complete resistance and partial resistance has not been made in this paper because so much variation in the degree of resistance was found. Sister animals produced by one or a few fissions from one animal frequently differed noticeably in the degree of slowing of movement and the progeny of a single resistant isolation line varied back and forth from one test to the next from clearly retarded to unaffected without any clear regularity.

INDUCTION OF RESISTANCE BY TREATMENT WITH ANTISERUM

In order to ascertain whether resistance to antiserum could be induced in stock 60 by exposure to antiserum, animals were put into various sublethal concentrations (1/200–1/1000) for 24 hours. The survivors were washed through two changes of culture fluid and either tested immediately with 1/40 or 1/80 antiserum or kept in culture fluid at 27°–29°C. Some of the products of division of the latter were tested for reaction after the first or second fission and every few fissions thereafter.

In the earlier part of the work, the animals used for exposure to antiserum were taken together with fluid from large mass cultures and mixed with sufficiently concentrated antiserum to give the desired final concentration of anti-

serum per animal. Preliminary observations had shown that the number of survivors was more closely related to the concentration per animal than to the concentration per unit volume. A few drops of the mixture of antiserum and animals were placed in each of a number of cavities in depression slides and observed after two hours and after 24 hours. In later work, five animals were placed with a micropipette into drops of antiserum which had been diluted to the desired concentration with salt solution. The latter technique has given more reproducible results than the former, but the reason for this is not clear.

As a result of work with the first method, it was reported by the writer (1947) that concentrations sufficient to immobilize all or nearly all of the animals but low enough to allow some survival of recovered animals after twenty-four hours yielded resistants most frequently, while lower concentrations in which many slightly affected animals survived yielded few if any resistants.

TABLE 1

The duration of antiserum-induced resistance in lines cultured at 27°–29°C.

DURATION IN NUMBER OF FISSIONS	NUMBER OF LINES			
	CLONE 1	CLONE 2	CLONE 3	TOTAL
1– 5	8	14	8	30
6–10	9	1	9	19
11–15	1	2	10	13
more than 15	0	1	8	9

Work with the second method has shown that this is not necessarily true. Approximately 80 percent of all the survivors of an exposure to 1/1000 antiserum were resistant. This concentration is just sufficient to slightly retard the movement of some of the animals but kills none of them. There were some indications that a still greater percentage of resistants was produced by treatment with 1/300 antiserum, but this could be attributed to differential mortality since some deaths occurred at this concentration. In 1/200 antiserum some animals which had been immobilized at two hours were found to have recovered after 24 and were then resistant to further exposure to antiserum.

Controls were run which demonstrated that neither salt solution by itself nor normal rabbit serum had any effect upon serum reaction. In one experiment, a small number of resistants appeared after exposure to salt solution; but a not significantly different number appeared among animals from the same culture kept continuously in culture fluid.

The resistant animals produced by either method transmitted the resistance to their descendants by fission. The length of transmission was determined by keeping the animals as isolation lines in culture fluid at 27°–29°C. Every few fissions a single animal was saved to continue the line and a part or all of the others were tested with 1/40 or 1/80 antiserum. Thus the resistant line was kept without further exposure to antiserum and without selection of more resistant or more sensitive animals. The number of fissions over which the resist-

ance was transmitted is shown for the different lines in table 1. No permanently resistant lines were found, though some lines were not carried long enough to be sure that they would revert.

It is certain that in the majority of these cases neither the induction of resistance nor its subsequent loss was associated with autogamy. The animals used to induce resistance had been through autogamy at most some ten days before, usually even less. Autogamy in this stock rarely occurs sooner than two weeks after a previous autogamy. In addition, staining checks have revealed little if any autogamy during the induction period. The lines which were followed for transmission were checked carefully for fission rate, and a drop in fission rate which should accompany autogamy was not found except in a few cases.

INDUCTION OF RESISTANCE BY TREATMENT WITH TRYPSIN

It seemed possible that the resistance induced by antiserum was the result of removal or inactivation of antigen molecules by the antibodies of the serum. To test this hypothesis, an attempt was made to remove the antigens by exposure to the proteolytic enzyme trypsin which LANCEFIELD (1943) reported removed the M-antigens from streptococci.

"Difco" trypsin (1:250) was dissolved in the same salt solution previously described. Some of the material failed to go into solution in the concentrations used, but this material was not removed. After a number of trials, concentrations between 0.08 and 0.06 percent were found most satisfactory. In somewhat higher concentrations most of the animals died, while in lower concentrations no clear effect was obtained.

Just as for the antiserum treatment, two methods af adding the animals were tried. In one, a trypsin solution twice the desired strength was mixed with an equal volume of fluid and animals from a mass culture of paramecia (stock 60). This mixture was dispensed by drops to depression slides. With the other method, trypsin of the desired concentration was made up in salt solution and five to ten animals were added by a micropipette to drops of this solution. With either method, the animals were allowed to remain in the trypsin for 24 hours. Some death always occurred in this time. The survivors were washed through two changes of culture fluid and either tested immediately with 1/40 antiserum or kept as isolation lines in culture fluid. Controls were always run using a trypsin solution which had been kept in a boiling water bath for two minutes to inactivate it.

The experiments using the first method of adding animals have yielded quite a few resistant animals, though there have been great variations in the percent produced in different trials. The experiments set up using the second method have yielded only a few slightly resistant animals. The figures for all experiments taken together are as follows. Of 181 animals isolated from trypsin, 98 were resistant to 1/40 antiserum. Of 191 animals treated with heated trypsin, none were resistant.

While it seems probable that the trypsin acted by virtue of its proteolytic activity, it should be pointed out that it had a distinctly injurious effect upon

the animals. Some animals were killed, and most of the rest failed to divide during the twenty-four hours in the enzyme. The animals in heated trypsin were not killed and divided two or three times during the twenty-four hours. The production of resistance cannot be attributed simply to the suppression of division, since animals which were prevented from dividing by starvation did not become resistant. However, the considerable effect of the trypsin upon the physiology of the animals makes it possible that the resistance arises as a result of some indirect effect.

The trypsin-induced resistance was transmitted for varying numbers of fissions when the trypsin treated animals were kept in culture fluid at 29°C.

TABLE 2

The duration of trypsin-induced resistance in lines cultured at 27°–29°C.

DURATION IN NUMBER OF FISSIONS	NUMBER OF LINES			
	CLONE 1	CLONE 2	CLONE 3	TOTAL
1– 5	0	1	1	2
6–10	2	0	4	6
11–15	2	0	7	9
more than 15	0	0	18	18

The methods of studying transmission were the same as those described in the previous section. The results are shown in table 2. The clones in table 2 are not the same as those in table 1.

RESISTANCE APPEARING DURING CULTURE AT LOW TEMPERATURE

In the course of an experiment designed to test the effect of temperature on the duration of antiserum-induced resistance, it was found that the resistance failed to disappear at all when the resistant lines were kept at 14°C, although sister lines kept at 29°C lost the resistance in typical fashion. This suggested the possibility that culture at 14°C without any previous treatment might convert sensitive lines into resistant ones.

Animals of 48 clones of stock 60 and in some cases several animals from the same clone were isolated and kept at 14°C as isolation lines. Periodically, the animals left over after the transfer of one animal to continue the line were tested with 1/40 antiserum. Thus no selection of resistant animals was practiced, and the animals used to continue the line were never exposed to antiserum. In every case, such lines became resistant and generally remained so on further culture at 14°C. The number of fissions that elapsed between the time at which the animals were put at low temperature and the time at which they became resistant is shown in table 3. The great variability shown cannot be adequately accounted for at the moment. While some of the variability may be attributable to differences between the 48 clones, there were marked differences between lines of the same clone. For example, the one line which did not become resistant until the 147th fission was one of 30 lines of a clone. The

other lines of the clone took from three fissions to 86, the majority taking less than 12.

In most cases, lines which had once become resistant remained so indefinitely as long as they were kept at 14°C (up to 178 fissions). A very few cases of temporary reversion to sensitivity were found. In all of these, resistance was regained after a few fissions. There was a great deal of variability within any particular line from one test to the next in the degree of resistance. Some animals were unaffected by two hours exposure to antiserum, while others were greatly slowed down but not immobilized. The general impression is that these lines had a varying amount of antigen per animal and that the amount averaged somewhat under that needed for immobilization.

TABLE 3

The number of fissions required for lines to become resistant when cultured at 14°C. Based on a study of 48 clones with from one to thirty lines per clone.

NUMBER OF FISSIONS AT 14°C BEFORE BECOMING RESISTANT	NUMBER OF LINES
1– 5	61
6– 10	47
11– 15	22
16– 20	9
21– 30	18
31– 40	9
41– 80	4
81–115	3
147	1

When resistant animals from lines kept at 14°C were placed at 27°–29°C, their descendants became sensitive again after varying numbers of fissions as shown in table 4. So far no clear differences have been found in the time needed to restore sensitivity in lines which had been kept for a short time (about 15 fissions) in the cold as compared with those kept there for longer periods (about 144 fissions), though there are some indications that those kept there for a longer period took somewhat longer. Four lines which had been kept in the cold for 134–158 fissions represent an exception. These lines remained resistant as long as they could be kept at 27°C. Three of these lines died after 14, 22, and 38 fissions respectively. They cannot be considered to have been carried long enough to show that they would not have become sensitive eventually. A fourth line remained sensitive for 119 fissions before it finally died. It seems possible that the failure to revert to sensitivity was correlated with the long time since previous autogamy, since another line of about the same age reverted to sensitivity after four fissions at 27°C but after 74 fissions at 27°C became resistant again and remained so as long as it could be cultured (44 fissions). It is known from the work of SONNEBORN (1938) that prevention of autogamy for long periods has a number of effects upon the physiology of *P.*

aurelia, including a reduction of viability followed by death of the line. It seems possible that such aging processes could also affect the production of antigens. In any case, there is no conclusive evidence that culture at 14°C any more than exposure to trypsin or antiserum is capable of producing permanently resistant lines.

DISCUSSION

The first question that needs to be answered is whether the changes reported here were the result of selection of spontaneously occurring changes as HARRISON and FOWLER (1945) believed was true for their material or whether the changes were induced by the treatments employed. It seems obvious that the latter was the case. The high percent of resistant animals which appeared following antiserum treatment in the absence of any possibility of selection taken in conjunction with the almost complete absence of resistant animals in untreated material is sufficient to demonstrate the point. This does not mean that spontaneous changes in antiserum reaction cannot occur in *P. aurelia* but merely that they were not an important factor in the present investigation.

TABLE 4

The number of fissions required for resistant lines arising at 14°C to become sensitive when cultured at 27°–29°C. Based on a study of 34 clones with from one to fifteen lines per clone.

NUMBER OF FISSIONS AT 27°–29°C BEFORE BECOMING SENSITIVE	NUMBER OF LINES
1– 5	36
6–10	8
11–15	5
16–20	5
21–25	4
26–30	7
31–35	4
36–40	6
40–73	5
113	1
not sensitive at 119	1

The next question to be answered is what part of the cell is modified as a result of the treatment. As JOLLOS (1921) pointed out, the purely transitory nature of the changes argues against gene mutation. The demonstration in the present investigation that these changes regularly disappear during vegetative reproduction adds strong support to this view and shows that mendelian recombination of mutant genes could not be involved.

Further support for the view that gene mutations are not concerned comes from a consideration of the nuclear constitution of *P. aurelia* and of the mutation rates that would have to be postulated. In this species, there are two

diploid micronuclei and a macronucleus which SONNEBORN (1940) has shown to be a compound structure probably composed of many diploid units. A gene mutation to have an immediate effect would have to be dominant and would probably have to occur in the macronucleus and therefore either occur simultaneously in several units or be dominant over many unmutated alleles of itself. This last statement is based upon the belief that the characters of Paramecium are directly dependent upon the macronuclear rather than the micronuclear genes (see SONNEBORN, 1947). The mutation would have to be induced both by antiserum and by trypsin with a frequency much higher than that known for any mutation-inducing agent, and it would have to be so unstable that back-mutation occurred with a relatively high frequency at 27°C. Finally, the rapidity with which resistance appeared following antiserum or trypsin treatment (24 hours, 0–3 fissions) would be very difficult to explain, especially since SONNEBORN (1943a) has shown that antigens can still be detected four to eight fissions after the genes necessary for their production have been lost as a result of recombination at autogamy or conjugation. The gene mutation hypothesis can thus be ruled out as a reasonable possibility.

There are left only the possibilities of a change in some cytoplasmic component or a change in some non-genic component in the nucleus. The fact that resistance was gained and lost in the absence of the micro- and macronuclear changes associated with autogamy and conjugation makes the second possibility somewhat less likely, though it does not completely exclude it. It can further be argued that it is unlikely that either trypsin or antiserum would penetrate much beyond the surface layers of the animal. On the other hand, the rapid attainment of resistance following treatment with agents that would be expected to affect the surface antigens suggests that the resistance was the result of removal or inactivation of these antigens. Thus there is suggestive evidence that the first step in the production of transmittable resistance is the removal or inactivation of a cytoplasmic component, the antigen.

How far can a loss of antigen from the surface account for the retention of antiserum or trypsin induced resistance for ten, fifteen, or more fissions? A simple removal would not be expected to lower the amount of antigen formed per fission, and so nearly normal amounts of antigen should be restored within a few fissions. This follows from the fact that untreated lines retain sensitivity for many fissions, perhaps indefinitely, and therefore enough antigen must be formed in each fission interval to supply a new animal with a normal amount. Thus if all antigen were removed, the products of the first fission after removal should have approximately $\frac{1}{2}$ the normal amount; the products of the second fission, $\frac{3}{4}$: the products of the third $\frac{7}{8}$, etc. The relations can be expressed in the equation $a_n = [a_0 + (2^n - 1)\, a_i]/2^n$ where a_n = amount per animal after n fissions, a_0 = amount at start (0 in the above calculations), and a_i = amount formed per fission (independent of a_0 and a_n by hypothesis). Since resistance is frequently maintained for ten, fifteen, and even more fissions, it seems almost inconceivable that this simple mechanism could be involved.

The assumption has been made in the preceding paragraph that there is equal distribution of antigen at each fission. While it is not likely that the dis-

tribution is really exact, the number of fissions needed for restoration would be only slightly affected except by very unequal distribution, and the fact that spontaneous resistance is so rare indicates that this cannot happen often enough to be an important factor.

The conclusion from the above discussion seems to be that exposure to antiserum or trypsin does lower the rate of formation of antigens subsequent to the treatment. There is, however, one other possibility that needs to be considered. The antigens might be restored to nearly normal concentration within a few fissions but might be unable to regain rapidly a position on the surface of the animal where they could be involved in immobilization. Since at each fission approximately half the surface of each animal is newly formed, this implies either that the surface is self-duplicating (a hypothesis so similar to one to be presented below that it will not be further considered) or that some other substance comes to occupy the surface when the antigens involved in immobilization are lost and somehow interferes with the occupation of new surface areas by the newly formed antigens. A complete exclusion of this hypothesis cannot be made with the evidence at hand. With this hypothesis, it is rather difficult to account both for the slow loss of sensitivity at 14°C and for the apparent equilibrium which is finally attained at this temperature. For this reason, this hypothesis will not be considered further here.

This leaves us with the probable conclusion that the rate of formation of antigens is lowered as a result of trypsin or antiserum treatment. It might be held that these treatments remove in addition to the antigens some precursor necessary for their formation. If this precursor had to be restored to 31/32 of normal concentration before any antigens could be formed and if the antigens in turn had to be restored to 31/32 of normal before an immobilization reaction could occur, then about ten fissions would elapse before sensitivity would be regained. If there were several steps between precursor and antigen, restoration of smaller fractions of normal concentration for each step would be needed in order to restore sensitivity in ten fissions. By assuming high enough thresholds and enough steps, retention of resistance for any number of fissions could be explained. This hypothesis has the drawback that it becomes rather complicated for resistance lasting fifteen fissions and more and has difficulty in accounting for the effect of culture at 14°C. As is shown in table 3, the loss of sensitivity at this temperature requires quite a few fissions. Culture at this temperature cannot, therefore, cause an immediate and complete destruction of antigens and precursor. Rather it must lower the net rate of production of one or both per fission either by partial destruction of that which is formed or by a reduction in the rate of formation. With the hypothesis now being considered, the reduction in amount of antigen, precursor, or both should follow the equation given on page 494, a_i becoming the net amount produced per fission interval at 14°C and a_0, the amount present in the sensitive animals prior to culture at 14°C. In consequence, a_n should rapidly approach a_i as a limit and a_i would have to be a quantity too small to maintain sensitivity. As in the case of return to sensitivity after loss, the assumption that there are several intermediates between a precursor affected by temperature and the anti-

gens would allow the loss to stretch over somewhat more fissions. However, the most favorable circumstance for long retention of sensitivity in respect to thresholds would be just the reverse of that for long retention of resistance; that is low threshold values. Because of the complicated assumptions that are needed to make this hypothesis fit the experimental results, it seems improbable and will not be considered further.

The simplest hypothesis and the one which most readily explains the facts is that the antigens involved in immobilization or some precursor of them are self-duplicating or in some other way facilitate the production of more of themselves. The removal or inactivation of the antigens or their precursors would be expected to lower the rate of formation. Retention of resistance for many fissions could be accounted for by appropriate assumptions concerning the relation between amount of substance present and the amount formed per fission interval.

There is a close similarity between this hypothesis and the one which SONNEBORN (1947) and PREER (1946) have developed to explain the inheritance of the killer character in the B group of varieties in *P. aurelia*. In both cases, it is assumed that a cytoplasmic material is present which is involved in the production of more of itself. In both, temporary but transmittable changes in the character concerned can be produced by procedures which reduce the amount of this cytoplasmic material in the animal.

There is one major difference between the results. By the methods which they employed, SONNEBORN (1947) and PREER (1946) were able to produce permanently non-killer lines from killer animals. In the present investigation, permanent resistance to antiserum has not been demonstrated even after prolonged culture at 14°C. This fact has made it impossible to make calculations of rate of multiplication of the cytoplasmic factors since the frequency of complete loss of these factors could not be ascertained. The failure to obtain permanently resistant animals was to be expected in view of the previously known differences between the A and B groups of varieties. In the former group, the clones derived from a conjugant pair become alike in phenotype in correspondence with their identical genotypes. Cytoplasmic differences which may have existed between the conjugants play no lasting role in determining the phenotype. For this reason, the presence of semiautonomous cytoplasmic factors has not up to now been shown in this group. On the contrary, in the B group of varieties, the two clones derived from a conjugant pair generally retain the phenotype of the parent stock from which their cytoplasm was derived despite the fact that it can be shown that they have identical genotypes.

There are three hypotheses that might be advanced to explain the differences between the two groups of varieties. (1) The A group of varieties may lack cytoplasmic factors of the sort found in the B group. (2) Cytoplasmic factors whose production cannot be initiated by genes may occur in both groups, but in the A group cytoplasmic transfer regularly occurs between conjugants whereas in the B group it occurs only under special circumstances. (3) The genes in the A group are able to initiate the production of cytoplasmic factors whereas those in the B group cannot. The present paper presents

strong evidence against the first interpretation. The second accounts for the similarity between exconjugant clones but offers no explanation for the failure to obtain lines permanently resistant to antiserum. The third hypothesis has already been advanced by SONNEBORN (1947). It will account for the failure to obtain permanently resistant lines as well as for the similarity of exconjugant clones in the A group. It implies for the present case that the antigens or their precursors are liberated into the cytoplasm by the genes. These cytoplasmic antigens or their precursors then accelerate the production of more of themselves either by self-duplication or some other process.

Can this hypothesis also explain the effects of culture at 14°C? The facts to be explained are the long time in terms of number of fissions necessary for loss of sensitivity, the similar long time needed for return of sensitivity at 27°–29°C, the failure to obtain permanently resistant lines after prolonged culture at 14°C, and the apparent establishment of an equilibrium in antigen concentration somewhat below the level needed for immobilization. On the hypothesis just presented, a certain amount of antigen would be formed per fission interval by gene initiation in the complete absence of any in the cytoplasm. An additional quantity would be formed per fission interval depending upon the amount of semiautonomous factor (antigen or precursor) present in the cytoplasm. A reduction in the first quantity by culture at 14°C would lead to a lowered amount of antigen, but an equilibrium point should be reached in a few fissions in accordance with the reasoning presented earlier since this amount would be independent of the amount of antigen or precursor already present. A reduction in the amount formed semiautonomously at 14°C would lead to a reduction of the total amount of antigen present per animal if the amount formed in this fashion per fission were less than that necessary to keep pace with the doubling of the number of animals. The production of resistance might require a few or many fissions depending upon the exact rates involved. It should lead to the establishment of a new and lower equilibrium level of the amount of antigen per animal. This equilibrium level would be equal to the quantity formed by gene initiation alone if no additional antigen were formed as a result of the presence of antigen or its precursor in the cytoplasm, but it should be higher than this if any were formed in this second way. The amount of antigen could fall to zero and stay there only if gene initiation did not occur.

The exact relation between genes and cytoplasmic factors cannot be ascertained from the present work. It may be that the genes form cytoplasmic factors independently of the amount of these factors already present and that the cytoplasmic factors form more of themselves in the cytoplasm, this latter process being dependent upon concentration of the factors. On the other hand, it is possible that the cytoplasmic factors are entirely formed by gene action, the gene being able to form a certain quantity in the absence of any cytoplasmic factor but acting more efficiently when some is present.

So far no evidence has been presented that antigens in Paramecium are really under the control of genes. While *a priori* it would seem very likely that they were, there is some direct evidence on this point. Unfortunately, no direct

evidence has yet been obtainable for the particular antigens studied in this investigation. However, SONNEBORN (1943a) has demonstrated a single pair of alleles which controls the antigenic difference between two stocks of variety 1. The writer (unpubl.) has also obtained evidence for genic control of antigenic differences between two stocks of variety 1. More than one gene difference was probably involved in the latter case.

The question of whether other characters in the A group of varieties are dependent upon similar semiautonomously formed cytoplasmic materials cannot be answered with certainty, but there is some suggestive evidence that they are. JOLLOS' (1921) observations on "Dauermodifikationen" are suggestive though it is not clear that all the cases which he so classified are really examples of the same phenomenon, and it would have to be demonstrated in any particular case that he was really dealing with a group A variety. Another class of phenomena, "cytoplasmic lag", also lends some support to this view. JENNINGS (1940) pointed out that the duration of the lag in some cases was difficult to understand unless it were assumed that the cytoplasm had the power to reproduce itself to some extent independently of the genes.

Thus it seems probable that cytoplasmic factors similar to those found in the B group of varieties also occur in the A group of *P. aurelia* and are not just a peculiar feature of some varieties, though there is an important difference in that in the A group the factors can be gene initiated whereas in the B group they cannot. Taken in conjunction with work on cytoplasmic inheritance in various other organisms, this suggests that such factors are of widespread, perhaps universal, occurrence though their relations to the genes may be different in different organisms.

SUMMARY

1. Antiserum against stock 60 of variety 1 of *Paramecium aurelia* regularly immobilizes and kills animals of this stock.

2. Exposure of animals of this stock for 24 hours to sublethal concentrations of antiserum or to the proteolytic enzyme trypsin results in the production of a high proportion of animals resistant to the action of concentrated antiserum.

3. The descendants by vegetative multiplication of these resistant animals retain the resistance for periods ranging from one to more than fifteen fissions, but when the lines are carried long enough it is finally lost without the intervention of conjugation or autogamy.

4. Isolation lines maintained at 14°C instead of the usual 27°–29°C become resistant to antiserum after varying numbers of fissions. The resistance is retained when such resistant lines are returned to 27°–29°C for periods ranging from one to more than fifteen fissions. With one exception it was finally lost if the line was continued long enough.

5. The interpretation is developed that the resistance is produced by loss or inactivation of the antigens concerned in immobilization.

6. It is shown that the transmission of resistance for many fissions can be accounted for most readily by self-duplication or some kind of semi-auton-

omous formation of the antigens concerned in immobilization or some substance necessary for their formation.

7. It is pointed out that there is a close similarity between the results reported here for a stock of the A group of varieties and the studies on the killer character in the B group of varieties of *P. aurelia*.

8. The failure to obtain permanently resistant lines is shown to be in accord with the known differences in the phenomena of inheritance in the two groups of varieties and is consistent with the view that the genes in the A group can initiate the production of cytoplasmic factors whereas those in the B group cannot.

9. The evidence presented in this paper makes it probable that semiautonomously formed cytoplasmic substances are of general occurrence in *P. aurelia* and not just a special feature of the B group of varieties. This conclusion lends support to the belief that such substances are of widespread, perhaps universal, occurrence in organisms in general.

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AN ELECTRON MICROSCOPE STUDY OF ISOLATED CHROMOSOMES[1]

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THE limitation in the penetrating power of electrons is one of the most serious obstacles in the study of chromosomes by electron microscopy. Chromosomes are generally larger than 0.2μ in diameter and are nearly opaque to electron penetration. At various stages of the nuclear cycle, however, chromosomes change their size and shape. Dividing chromosomes are generally largest and "resting" chromosomes smallest. In order to take advantage of the small size of resting chromosomes, this study has utilized chromosomes from resting cells.

A second serious obstacle to the use of electron microscopy in the cytology of chromosomes is the need for drying the material. In the process of drying, changes take place in the chemical and physical nature of the medium which surrounds the chromosomes and this seems inevitably to alter their structure. Air drying has not been found practical without previous cytological "fixation." The method of freeze-drying has been tried but has not been found practical. In the air-drying of fixed material, some slight change of surface form seems inevitable, such as ripple-marks made by the decreasing size of the drop of water upon the soft surface of the chromosome.

The internal structure of chromosomes as indicated by genetic deduction and cytological observation suggests a finer structure which might be discernible by electron microscopy. The opaque nature of the chromosome, due to its large size and impregnation with fixatives, compels one to study the finer structure by means of controlled decomposition of the body.

MATERIAL AND METHOD

The chromosome material used was from the nuclei of chicken red blood cells. The chromosomes of these cells were isolated by a method similar to that described by POLLISTER and MIRSKY (1942). The method used by CLAUDE and POTTER (1943) for leukemic cells would probably also have been satisfactory. The red blood cells are washed in 0.14 Molar NaCl (physiological saline) and are then physically broken up in a high speed blendor (the Waring blendor was used). A short period of action of the blendor may break the cells and leave the nuclei intact. A longer period may rupture the nuclear membrane and free the chromosomes. By either method the chromosomes may be centri-

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Magnifications given in this paper are subject to an error of about ten percent due to fluctuations inherent in the instrument.

fuged out and the cytoplasmic materials washed away. A repetition of this process will leave a large quantity of chromosome threads as a residue. These strands are visible by ordinary microscopy and may be fixed or stained by standard cytological procedures.

The chromosome threads may be dissolved in 1 M NaCl, precipitated into strands by dilution down to 0.14 M NaCl, or may be denatured by still further dilution (as in distilled water). These characteristics of chromosomes were first described by POLLISTER and MIRSKY (1946) and MIRSKY and POLLISTER (1946). It has been shown that the chromatin threads isolated by this technique consist primarily of desoxyribose nucleoprotein.

The denaturation or dissolving of the chromosomes in nonisotonic salt solutions is a difficulty which must be overcome if one wishes to study whole chromosomes. For the study of the chromosome constituents, however, advantageous use may be made of these characteristics.

Fixation of the chromosome threads so as to prevent disintegration of form was accomplished by the use of various standard cytological fixatives. The form of the chromosome is different after treatment with each substance. Some of these differences are shown in the accompanying micrographs.

The chromosomes were fixed while they were in fine suspension in isotonic saline. In order to prevent clumping of the threads, shaking was avoided during fixation. If the chromosomes are fixed while clumped, observation of their structure as individuals is impossible for they can not be separated one from the other.

Various fixatives and combinations of these were tried. Osmium tetroxide seemed to be the best for retaining what appears to be the natural shape. Acetic-alcohol seemed to shrink the chromosomes while chromic acid darkened them considerably. The illustrations show the comparative effects of the three fixatives. Clumping was enhanced by the acetic-alcohol and was least apparent with chromic acid.

After fixation, the chromosomes were allowed to settle. To avoid their clumping together permanently, centrifugation should not be employed at this time. They can then be transferred to distilled water and a drop of suspension dried upon a collodion electron microscope screen. If desired, any chromosome may be located with the light-optical microscope upon the screen before observation with the electron microscope. Micrographs of these chromosomes may be obtained directly or they may be shadow-cast The method of shadow-casting allows one to determine the third dimension of electron microscope views.

In addition to the whole chromosomes studied in this way, mounts were made of the material precipitated from solution of chromosomes in 1 M NaCl and subsequently diluted. For the preparation, no fixative was employed. A drop of the viscous solution was placed upon the screen, diluted to precipitate the chromosome material, and then dried. The material precipitated in this way may be observed with the ordinary microscope and is found to consist of numerous anastomosing strands. They may be stained with acetic-orcein for ordinary observation but need not be fixed or stained for electron microscope study.

OBSERVATIONS

Chromosomes isolated from chicken red blood cells are from 0.2μ to 0.4μ in diameter and range to several microns in length. Their shape is variable and differences in surface form are apparent. Most of the latter variability seems to depend largely upon the fixative employed. With the light microscope, the chromosomes appear as fine strands of the same diameter and some appear to have an appendage at one end. This appendage may be in the nature of a satellite.

Figures 4 to 8 show some chromosomes of the chicken red blood cells. Figure 4 shows a chromosome strand fixed with osmium tetroxide followed by acetic-alcohol. It is about 0.3μ in diameter and about 7.0μ in length (only about half its length is shown). The body seems to consist of a central core (shown white) surrounded by protuberances which may or may not be flattened around the sides. These protuberances are spaced in such a way as to indicate a spiral around the central core. The "shadow" produced by the chromium layer indicates that the protuberance extends up and down as well as laterally. Nevertheless, the body of the chromosome has a flattened appearance, probably produced by the pressure of drying.

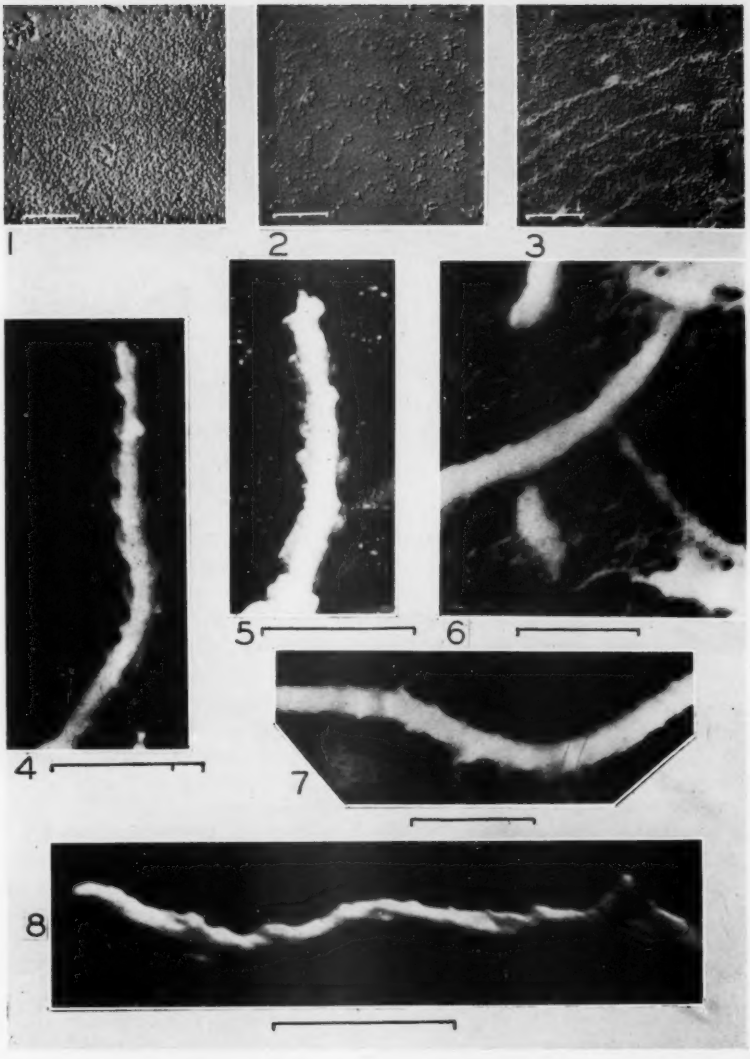
Figure 5 shows another chromosome fixed in the same manner. The protuberances do not appear as large in size nor as prominent as in figure 4. Nevertheless, they show a spiral. The production of prominent protuberances seems to be dependent upon the fixative used. Chromosomes fixed with osmium tetroxide or chromic acid alone show the protuberances only very slightly.

Figure 6 shows portions of four different chromosomes fixed with osmium tetroxide. A small end-portion at the top does not indicate much structure. The large chromosome curving across the center demonstrates small protuberances along the sides. A very narrow chromosome of less than half the usual diameter lies under it, curving down to the right. Beneath this can be seen the end of a chromosome of the normal width with an appendage attached. The appendage is of the normal diameter (0.3μ) but the attachment thread is considerably smaller (0.065μ or 65 mμ). The length of the appendage is about 0.9μ and its attachment thread is also about 0.9μ.

Figure 7 exhibits a portion of a long chromosome fixed with chromic acid alone. Characteristic of chromosomes fixed with chromic acid is the complete opacity of the material to electron penetration. Also, the protuberances along the sides are quite small by comparison with acetic-alcohol fixed chromosomes. If one were to connect the positions of the protuberances with an imaginary thread, it would be apparent that these protuberances could be attributed to a thread coiling within or upon the chromosome body and showing its position by surface bulges.

Figure 8 shows a somewhat narrower chromosome fixed with osmium tetroxide alone. It exhibits a twisting of the body, a series of surface protuberances of the same nature as described above and surface marks all in the same direction; the latter can probably be attributed to the effects of drying.

Figures 1 to 3 illustrate various stages in the formation of strands after the chromosomes have been dissolved in 1 M NaCl. Figure 1 shows evenly-spaced



FIGURES 1–3.—Particles obtained by dissolving isolated chromosomes in M NaCl, diluting with water and then drying. Various degrees of strand formation are apparent, occasioned by different degrees of clumping of the particles. Note the spacing of the clumps in fig. 2.

FIGURES 4 and 5.—Portions of chromosomes fixed with osmium tetroxide while in physiological saline and then followed by acetic-alcohol.

FIGURE 6.—Portions of several chromosomes fixed with osmium tetroxide.

FIGURE 7.—Portion of a chromosome fixed with chromic acid.

FIGURE 8.—An entire chromosome fixed with osmium tetroxide.

The scale for all the figures is one micron. All have been shadow-cast with chromium.

spheres having diameters of about 50 mμ, figure 2 shows the beginning of a clumping of the spheres and figure 3 shows a later stage It is clear from figures 2 and 3 that the strands precipitated by dilution are formed from spheres of the same diameter as in figure 1 by a clumping action. Also, it is interesting to note that the clumps themselves are spaced evenly over the field (Fig. 2). This predisposition of the clumps may imply the existence of forces acting at a distance between the spheres. On a larger scale, the spacing can be observed in a test tube between gross particles of chromosomes which have not entirely dissolved when an excess of chromosome material is added to 1 M NaCl.

DISCUSSION

A much greater detail is apparent in the chromosome micrographs obtained by electron microscopy than is possible by ordinary microscopy. A series of protuberances or bumps are found around the chromosomes which seem to be attributable to an internal spiral nature of the chromosome or to a spiral thread within a matrix. This spiral nature is most apparent when the chromosomes are fixed with acetic-alcohol.

ELVERS (1941, 1943) has shown micrographs of pachytene chromosomes of Lilium (not isolated) which are similar to the "resting" chromosomes here described fixed with chromic acid. ELVERS (1941) was not certain whether the protuberances were chromosomal in nature or whether they were cytoplasmic. It seems likely that cytoplasm was attached to lateral protuberances in his material. Owing to the lack of isolation from cytoplasm, ELVERS' pictures are not very clear and only one shows a distinctness approaching those here presented. That one (Plate VIII, fig. 66) shows that the structure of the pachytene chromosome of Lilium pollen mother cells is not very different from the structure of the resting chromosome of the chicken red blood cells.

Recently, BUCHHOLZ (1947) has shown some electron micrographs of maize pachytene chromosomes which are different from those described by ELVERS and those described here. Little detail is shown and BUCHHOLZ does not seem cognizant of ELVERS' previous work. CLARK, BARNES and BAYLOR (1942) published a note on an electron microscope study of chromosomes but it seems indecisive on all points.

In none of this work has internal detail of chromosome structure been discernible. This may be attributable to opacity of the chromosomes to electrons and to the subsequent scattering of the latter. It is possible that a picture similar to the pachytene chromosomes of BUCHHOLZ may be obtained by means of a greater longitudinal stretch of the chromosome provided that the bulges observed in the resting chromosomes are the "chromomeres" of the pachytene which are attached to a coiled thread. No other sign of the existence of "chromomeres" is apparent in the chicken resting chromosomes. If this is true, a greater coiling for the resting chromosomes than the pachytene chromosomes would be indicated. Also, the definite existence of both gyres of spirals, and of chromomeres is implied. Definite information on chromomere structure is certainly needed.

These pictures of isolated chromosomes give the following new information:

a definite spiral structure is apparent in the resting chromosomes which may be in the form of a thread imbedded in a matrix, no chromomeres can be observed in the resting chromosomes, and appendages can be clearly seen and photographed with their attachment threads.

The dissolved and precipitated chromosome material gives a further indication of the nature of the chromosome threads. The method of precipitation or clumping of the small spheroidal particles indicates that in high salt concentration these particles are spaced farther apart but bear a geometrical relationship to one another. In physiological saline, complete clumping occurs. After treatment in distilled water, the material is denatured and will no longer dissolve or reform into strands at will. The denatured material appears as dark clumps with no definite shapes by electron microscopy.

Through the courtesy of DR. J. P. GREENSTEIN, purified thymus nucleic acid has been obtained for electron micrographs. The material has been found not to form particles nor strands similar to those of nucleoprotein shown in figures 1 to 3. The clumping and strand formation therefore seems to be a characteristic of the complete nucleoprotein combination.

At present, there does not seem to be any reason for speculating on the structure of the particles or on their relation to "matrix," chromonemata or the gene.

SUMMARY

Chromosomes were isolated from the resting nuclei of chicken red blood cells by fragmentation and centrifugation of the cells in physiological saline. They were fixed with ordinary cytological fixatives and studied by electron microscopy. It is found that the resting chromosomes are not very different from pachytene chromosomes of Lilium shown by ELVERS. A definite spiral structure is apparent in the chromosomes which may be in the nature of a thread imbedded in a matrix; appendages have been seen on the chromosomes but no definite chromomeres are apparent. Chromosomes, dissolved in M NaCl and precipitated, form strands composed of minute particles about 50 mμ in diameter. Purified thymus nucleic acid does not show this formation when treated in the same manner.

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GENE RECOMBINATION AND LINKED SEGREGATIONS IN ESCHERICHIA COLI[1]

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THE occurrence of factor recombination in the bacterium, *Escherichia coli*, has been described in previous reports (LEDERBERG and TATUM, 1946 b, c, TATUM and LEDERBERG, 1947). In an attempt to elucidate further the genetic structure of this organism, these studies have been extended to crosses involving several characters, and to the quantitative enumeration of various recombination classes. The results described in this paper provide evidence supporting the sexual basis of factor recombination and of the existence of an organized array of genes comparable to that of higher forms.

MATERIALS AND METHODS

The parent "wild-type" strain, K-12, of *E. coli* used in these experiments and the production and behavior of biochemical mutants have been described (GRAY and TATUM, 1944, LEDERBERG and TATUM, 1946a, ROEPKE, LIBBY, and SMALL, 1944, TATUM, 1945). Specific requirements, notation, and other data pertinent to the biochemical mutants are summarized in tables 1 and 2. In general, a biochemical deficiency resulting from mutation is designated by the initial of the substance required (e.g. B^- for biotinless), while the wild type alternative is written with a "+" sign (e.g. B^+ to emphasize the alternative to B^-). The term "prototroph" (RYAN and LEDERBERG, 1946) has been devised for strains exhibiting the nutritional behavior of the wild type, which for *E. coli* implies independence of any specific growth factors. Prototroph is, however, not synonymous with "wild type" since it refers (a) only to the phenotypic appearance of a culture and (b) only to nutritional and not to other possible mutant characteristics.

K-12 as a coliform is capable of fermenting, or producing acid, from a variety of sugars, including glucose, galactose, maltose, lactose and mannitol; however, it ferments glycerol only weakly, and sucrose even less so. Because of the ease of scoring and their biochemical specificity, mutants unable to ferment various sugars have been looked for. Particular attention was paid to the isolation of "lactose-negative" or "*Lac*$^-$" mutants, because of the taxonomic significance which has been attached to this character.

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The detection of fermentation mutants is readily accomplished by the use of indicator media. The medium "EMB-lactose" used in routine bacteriological work was found to be highly useful. It consists of the following (in g/l): peptone (or "N-Z-Case") 10, yeast extract 1, lactose 10, agar 15, eosin Y 0.4, methylene blue 0.06, sodium chloride 5, dipotassium phosphate 2. On this medium, colonies of bacteria which can ferment lactose (or any other sugar added in its place) rapidly turn a deep purple color, while colonies of non-fermenting organisms remain white or pink but may slowly turn light blue.

Lac$^-$ mutations have been recovered in two instances. Among 15,000 colonies

TABLE 1

Symbols used for various loci.

1. Nutritional requirements. Allele for requirement of a given substance is designated by the superscript "$^-$"; independence by "$^+$". E.G., B^- is biotinless; B^+ is biotin-independent.

B	biotin	*L*	leucine	*Pa*	phenylalanine (ϕ was used previously, but has been modified for typographical reasons)
B_1	thiamin	*M*	methionine		
C	cystine	*P*	proline		
		T	threonine		

2. "Sugar" fermentations. The ability to ferment is designated "$^+$"; the inability "$^-$".

Lac	lactose	*Gly*	glycerol

3. Bacteriophage resistance. Resistance is designated by the superscript "^{r}"; sensitivity by "^{s}". E.G. $V_1{}^r$.

V_1	resistant to *T1*, *T5*
V_{1a}	resistant to *T1*; sensitive to *T5*
V_{1b}	resistant to *T1*; mucoid colonies
V_6	resistant to *T6*.

4. Resistance to chemical agents. Resistance and sensitivity "^{r}" and "^{s}" respectively, as *Cla*^{r}.

Cla	sodium chloroacetate
A	sodium azide

of strain Y-10 ($T^-L^-B_1^-$) obtained by spreading a culture previously treated with ultraviolet light on EMB-lactose agar, a single pink colony was noted. It proved to be the same, nutritionally, as Y-10 and was therefore regarded as a *Lac*$^-$ mutant; this stock is labelled Y-53. Among 30,000 colonies of Y-40 ($B^-M^-V_1{}^r$) a single *Lac*$^-$ was recovered following treatment with nitrogen-mustard (TATUM, 1946), and was designated as Y-87. Tests showing that these independent mutations are probably allelic will be described in a later section (see table 5). Strains Y-53 and Y-87 differ in the rate at which the *Lac*$^-$ character reverts to the *Lac*$^+$ condition, but whether this is due to different allelic states or to differences at other loci, cannot be definitively asserted.

Attempts to obtain maltose, mannitol, and galactose-negative mutants were not successful, presumably because the populations tested were too small. A glycerol-negative strain has been obtained, but the wild type ferments this polyalcohol so poorly to begin with that accurate scoring is difficult; studies on this character will not be further reported here.

Mutations for resistance to specific bacteriophages or bacterial viruses have proven to be exceedingly useful. They are readily obtained as spontaneous mutants by plating a large number of sensitive bacteria with the particular virus in question; only resistant mutants escape lysis and may be recovered as "secondary" colonies (fig. 1). Resistant mutants are readily freed from residual virus by serial single colony isolation. Resistance to a given virus may be

TABLE 2

A summary of the mutants used.

STRAIN NO.	GENOTYPE	ORIGIN	GENOTYPE	AGENT
K-12	prototroph.	Original wild strain		
58	B^-	K-12	B^+	X-ray
58-161	B^-M^-	58	B^-M^+	X-ray
58-278	B^-Pa^-	58	B^-Pa^+	X-ray
Y-24	$B^-Pa^-C^-$	58-278	$B^-Pa^-C^+$	ultra-violet
679	T^-	K-12	T^+	X-ray
679-680	T^-L^-	679	T^-L^+	X-ray
Y-10	$T^-L^-B_1^-$	679-680	$T^-L^-B_1^+$	X-ray
Y-46	$T^-L^-B_1^-V_1^r$	Y-10	$T^-L^-B_1^-V_1^s$	selection
Y-53	$T^-L^-B_1^-Lac^-$	Y-10	$T^-L^-B_1^-Lac^+$	ultra-violet
Y-64	$T^-L^-B_1^-Lac^-V_1^r$	Y-53	$T^-L^-B_1^-Lac^-V_1^s$	selection
Y-40	$B^-M^-V_1^r$	58-161	$B^-M^-V_1^s$	selection
Y-87	$*B^-M^-V_1^rLac^{z-}$	Y-40	$B^-M^-V_1^rLac^+$	nitrogen mustard
Y-24-V_1^r	$B^-Pa^-C^-V_1^r$	Y-24	$B^-Pa^-C^-V_1^s$	selection
679-183	T^-P^-	679	T^-P^+	X-ray
Y-88	$T^-L^-B_1^-Lac^-Cla^r$	Y-53	$T^-L^-B_1^-Lac^-Cla^s$	selection
Y-80	$B^-M^-V_1^r$ *Gly*$^-$	Y-40	$B^-M^-V_1^r$ *Gly*$^+$	nitrogen mustard
Y-91	$B^-M^-V_1^r$ *Cla*^{r}	Y-40	$B^-M^-V_1^r$ *Cla*^{s}	selection
Y-92	$B^-M^-V_1^rAz^r$	Y-40	$B^-M^-V_1^rAz^s$	selection
Y-94	$T^-L^-B_1^-Lac^-V_6^r$	Y-53	$T^-L^-B_1^-Lac^-V_6^s$	selection
Y-100	$T^-L^-B_1^-Lac^-V_{1a}^r$	Y-53	$T^-L^-B_1^-Lac^-V_{1a}^s$	selection
Y-86	$T^-L^-B_1^-Lac^-V_{1b}^r$	Y-53	$T^-L^-B_1^-Lac^-V_{1b}^s$	selection

* Lac^{z-} in mutant Y-87 differs from Lac^- in mutant Y-53 and its derivatives in the greater reverse-mutability of the latter. Lac^- and Lac^{z-} are otherwise similar, and allelic.

scored by streaking a loopful of bacteria on an EMB or nutrient agar plate at right angles to a previous streak of the virus suspension (DEMEREC and FANO, 1945, see fig. 1 of the present report.)

It was found, however, that mutations for resistance to a given virus are not entirely specific, but that resistant mutants display "cross-resistance," i.e., are also resistant to other viruses. For example, most $T1$-resistant types are also resistant to $T5$. (For the nomenclature of the bacterial viruses used in this investigation, and a detailed account of the cross-resistance patterns of another strain, *E. coli B*, see DEMEREC and FANO, 1945). The cross-resistance patterns of K-12 are similar to those of *E. coli B* with the exception that $T1$-resistant mutants which are sensitive to $T5$ are not tryptophaneless, as has

been reported by ANDERSON (1946) for the corresponding mutants of *E. coli B*. In this paper, the designation V_1^r will be used for the more frequent T_1-resistant mutant, which is also resistant to T_5. The symbol V_{1a}^r is reserved for the T_5-sensitive, T_1-resistant mutant, but the evidence that distinct loci are involved will be presented *in extenso* in another place.

In addition to V_1^r and V_{1a}^r, just mentioned, a third type of "secondary colony" has been found among populations treated with the virus T_1. This type, V_{1b}^r is characterized by an exceedingly slimy or mucoid colony confor-

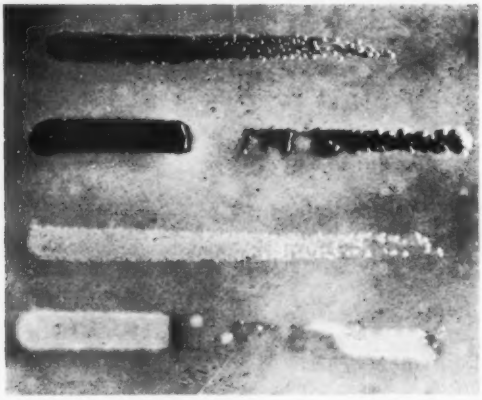


FIGURE 1.—The phenotypes of the four combinations of *Lac* and *V* are illustrated. In order they are: $Lac^+V_1^r$; $Lac^+V_1^s$; $Lac^-V_1^r$; $Lac^-V_1^s$. An EMB-lactose agar plate was first streaked vertically with the virus T_1. Subsequently, each of the bacterial types was streaked, from left to right, perpendicularly across the virus streak. After 16 hours incubation, both the *Lac* and V_1 phenotypes are well developed. Developing in the zone where $Lac^-V_1^s$ has been lysed can be seen two colonies of resistant mutants: $Lac^-V_1^r$.

mation. Recombination studies on this mutant are complicated by its genetic instability; V_{1b}^r rapidly reverts to the wild type, and in addition may also be strongly selected against in competition with V_{1b}^s. However, the locus of V_{1b} can be distinguished from the locus of the other V_1 mutants by the demonstration of a different recombination frequency with *Lac*. These data are summarized in order to emphasize the importance of genetic tests to insure the allelic identity of phenotypically similar mutants.

It is particularly fortunate that resistance tests can be conducted on EMB agar, since this allows the characterization of a strain with respect to virus-resistance and to lactose fermentation with a single streaking (see fig. 1).

Mutants resistant to sodium chloroacetate (Cla^r) were obtained by streaking a large number (about 10^7) of bacteria on nutrient agar to which filter-sterilized chloroacetate has been added to make a final concentration of 2 mg/ml. At this concentration, the wild type is substantially inhibited, while resistant mutants grow luxuriously. This mutation is accompanied by deficiencies in

the metabolism of pyruvic and acetic acids, which will be described in more detail elsewhere. Independent mutations to other inhibitors, including iodoacetate, azide, streptomycin, streptothricin, mercuric chloride, and Brilliant Green, can be secured in a similar fashion, but genetic analysis of these mutations has not been completed.

Morphological variation has occasionally been noted (exceedingly rough or very mucoid colonial form) but is relatively unsuitable for genetic work because the presumably random choice of prototroph recombinants may be influenced.

In addition to the EMB agar already described, a number of other natural or "complete" media have been used. The Difco product "Penassay Broth" has been used most extensively, and is satisfactory for the preparation of inocula, except that it must be supplemented with cystine for the growth of cystineless organisms, such as strain Y-24. Other satisfactory media include a broth consisting of: peptone 5, glucose 5, yeast extract 3, g/l, as well as Difco Nutrient Broth, and diverse concoctions containing peptone or casein hydrolysates and meat or yeast extract.

The synthetic or minimal medium contains, in g/l: NH_4Cl 5, NH_4NO_3 1, Na_2SO_4 2, K_2HPO_4 3, KH_2PO_4 1, glucose 5, asparagine 1.5, $MgSO_4$ 0.1, trace elements (GRAY and TATUM 1944), and $CaCl_2$, a trace. The medium is made solid by the addition of agar in a concentration of 1.5 percent.

To avoid flocculation when used with agar, the glucose and agar in solution should be autoclaved separately, and mixed with the other components just before using. Unwashed agar (Difco) is sufficiently free of the growth factors under consideration to be satisfactory for many experiments; the use of washed agar, however, is recommended for the cleanest results.

The detection of recombinants is based upon the inability of biochemical mutant bacteria to proliferate in the absence of their specific growth substances. Plating in minimal agar, therefore, has the effect of a sieve for prototroph cells. To insure against contamination with prototrophs derived by reverse mutation, which has been noticed at certain loci, it has been desirable to use multiple biochemical mutants as the parental stocks in recombination studies. Coincidental reversion at two or more loci is theoretically improbable, and experimentally undemonstrable (RYAN, 1946, TATUM and LEDERBERG, 1947). For example, plating either $B^-M^-T^+L^+B_1^+$ or $B^+M^+T^-L^-B_1^-$ separately into minimal agar did not lead to the appearance of prototrophs, $B^+M^+T^+L^+B_1^+$. When, however, a mixture of these cell types was so "sieved," one prototroph was found for about each 10^7 cells inoculated. These have been assumed to arise from the recombination of "+" alleles to form the prototroph.

In previous experiments, the two multiple mutants were inoculated together into a complete medium and allowed to grow in mixed culture before plating into minimal agar. This method is not satisfactory for present purposes because it allows possible selective differentials to alter the relative frequencies of different recombination classes. A modified procedure has been developed, which will now be described in detail.

The mutant stocks are maintained on "complete" agar slants, transferred

at intervals of 6–8 weeks. They are inoculated separately into test-tubes containing about ten ml of liquid complete medium and incubated overnight at 30°C with gentle shaking. The following morning, an additional ten ml of the same medium is added to each culture, and the tubes are incubated in the same manner for an additional three to five hours. These cultures contain from 1–4× 10^9 cells per ml. They are then washed in the following manner: the cotton plugs are replaced with sterile corks which have been kept in 95 percent alcohol and the alcohol flamed off just before using. The cultures are then centrifuged at about 2500 r.p.m. for 20 minutes, which suffices to pack the cells in the bottom of the test tubes. The supernatant medium is carefully poured off, and the tube is rinsed with about 10 ml sterile distilled water, care being taken not to disturb the pellet. The cells are then resuspended in an additional 15–20 ml sterile water, and recentrifuged. The supernatant wash water is decanted and replaced with an equal volume of fresh sterile water, in which the cells are suspended. In the meantime, minimal agar plates are prepared. A bottom layer of about 15 ml minimal agar is poured into each Petri plate and allowed to solidify. Cell suspensions of different mutant stocks are mixed at this time and measured quantities (usually about 10^8–10^9 cells) are pipetted onto the agar surface. At this time also, one may add such growth factor supplements as are desired to permit the growth of recombination types other than prototrophs. The cell suspensions are then mixed into a layer of about ten ml molten minimal agar (at 45–50°C) which is poured onto the plates. After the agar hardens, the plates are incubated at 30°C for a period of 48 hours. At this time prototroph colonies will be found distributed throughout the plate, many of them at or near the surface and accessible to picking for further characterization.

The procedure may be varied in several ways. It is important however that the inoculum consist of "young" cells, since cultures of 24 hours or older have given quite inconsistent results. It is possible to store the inoculum in distilled water for at least twenty-four hours without appreciably affecting the yield, which suggests that the aggregation of genetic types leading to the recombination process occurs in the molten or the solidified agar. This occurrence must, however, take place within a few hours, since the recombinant prototrophs are not appreciably slower to appear than wild type cells in a similar physiological state which may be streaked on the surface of the plates. Presumably, therefore, one could increase the yield of prototrophs by making conditions more favorable for the free contact of the cells, as by packing them together in a centrifuge tube in minimal liquid medium. However the complication of proliferation of prototrophs already formed would interfere with the interpretation of such an experiment. Many physiological factors may interfere with the recombination process, and, for example, the yield may be reduced markedly by inoculating too heavily, or by omitting an under-layer of agar into which, presumably, deleterious metabolic products may diffuse. Instead of mixing the cells in semisolid agar, it is possible to streak the mixture on the surface of slightly dried minimal agar plates. Under these conditions, however, the prototroph colonies are likely to be more heavily contaminated with the residual parental mutant types.

For most purposes, however, this contamination may be ignored, as will be shown in a later section. Prototroph colonies are then fished and streaked directly on EMB plates, or otherwise tested, to classify them with respect to other factors that may be segregating.

RESULTS AND CONCLUSIONS

In most organisms inheritance is studied by the examination of zygotes carrying the gene alternatives determining a character. The segregants are chosen at random, and factor linkage is recognized by deviations in the frequency of parental and new couplings of a series of characters. In the absence of a random method of separating zygotes in *E. coli*, one is limited here to the members of specific recombination classes, namely the prototrophs. It is however, possible to introduce other factor differences into the biochemical mutants from which prototrophs are obtained, and to determine how such factors segregate into this recombination class. It was hoped in this way to obtain information concerning the haploid or diploid condition of the bacterial cell, and to determine whether factors segregated at random, or according to specific, perhaps linear chromosomal laws.

The first factor pair to which this approach was applied was V_1^r/V_1^s (LEDERBERG and TATUM, 1946b). In the cross $B^-M^-P^+T^+V_1^r \times B^+M^+P^-T^-V_1^s$, ten $B^+M^+P^+T^+$ were isolated. Eight proved to be V_1^r while two were V_1^s. This at once suggested that the vegetative cell of *E. coli* is haploid, since segregation could be observed in the first filial generation clone. It was noted also at that time that the "reversed" cross: $B^-M^-P^+T^+V_1^s \times B^+M^+P^-T^-V_1^r$ gave quite a different ratio of r/s in the prototrophs, namely 3:7. Results on so small a sample are of doubtful significance, but they suggested the technique by which the basis of this character "segregation" could be elucidated. For this reason, the study of "reversed" crosses was extended to include numerically more data, using various combinations of mutants, and involving in addition to V_1^r/V_1^s, Lac^+/Lac^-. The information which was obtained is summarized in tables 3 and 5. The data show clearly that neither of the factor alternatives V_1^r/V_1^s or Lac^+/Lac^- segregates at random into the prototroph recombination class. However, the occurrence of all factor combinations, albeit with different frequencies, is evident, at least with respect to *Lac* and V_1. It seemed clear that there are only two alternative explanations for the unequal frequencies with which alternative alleles are manifested in the prototrophs: (a) that the alleles were characterized by some differential physiological property, such as dominance, or preferential segregation, or (b) that the nonrandom segregation was due purely to the mechanics of factor recombination, which is to say a linkage system.

The results of "reversed crosses" have a distinct bearing on this problem. If nonrandom segregation into prototrophs were due to some physiological property of the allele concerned, its particular coupling in the parent in which it is introduced should have no great effect on the segregation frequency; if on the other hand, the effect were purely mechanical, the segregation would reflect entirely the couplings of the parents, and the substitution of one allele for

another in the parents (as in reversed crosses) should lead to a corresponding inversion in the ratios with which that allele is found in the prototrophs. The tables cited show that in every case there is no agreement between the ratios found in reversed crosses, unless the comparison is made with one of the ratios inverted, in which case there is reasonably good agreement. This result is in accord with the hypothesis that the genes in *E. coli* are arranged in one or more linkage groups, and is in disagreement with the postulation of a diploid con-

TABLE 3

*Comparisons of V_1^r segregations when introduced with alternative parents.**

PARENTS			PROTOTROPHS [$B^+M^+Pa^+C^+T^+L^+B_1^+P^+$]		
			V_1^r	V_1^s	% V_1^r
$B^-Pa^-C^-T^+P^+$		$B^+Pa^+C^+T^-P^-$			
$\cdots V_1^r$	×	$\cdots V_1^s$	76	6	92
$\cdots V_1^s$	×	$\cdots V_1^r$	30	107	22
$B^-Pa^-C^-T^+L^+B_1^+$		$B^+Pa^+C^+T^-L^-B_1^-$			
$\cdots V_1^r$	×	$\cdots V_1^s$	80	23	77
$\cdots V_1^s$	×	$\cdots V_1^r$	53	133	28
$B^-M^-T^+P^+$		$B^+M^+T^-P^-$			
$\cdots V_1^r$	×	$\cdots V_1^s$	49	8	86
$\cdots V_1^s$	×	$\cdots V_1^r$	5	19	21

* See LEDERBERG (1947) for a statistical analysis of tables 3, 5, and 6.

dition, or with a state of indefinite "ploidy" which would be characteristic of a system of cytoplasmic inheritance.

The results of these experiments seemed sufficiently secure that one could adopt the existence of a linkage system as a working hypothesis and on this foundation, an attempt has been initiated to "map" a number of markers in *E. coli*. It was hoped at first that there might be found linkage groups which would be independent of one another, so that recombination between biochemical markers in one group could be used to detect recombinants, yet not interfere with the segregations in the other group(s). There was, however, no immediate prospect that these relationships could be found initially, so it was decided to study linkage relationships in a single pair of mutant stocks, and their derivatives. The stocks which were selected for this study were 58–161 (B^-M^-) and Y-53 ($T^-L^-B_1^-Lac^-$) and their V_1^r mutants. Since *Lac* and V_1 could be so readily scored, using only a single streak from each prototroph colony which appeared, it was hoped that the collection of an adequate volume of data could be accomplished with greater facility than if biochemical markers only were used.

It was, however, necessary to determine the relationships of the biochemical mutant loci of which at least four must be used to obtain recombinants. Mixtures were, therefore, plated into minimal medium supplemented with a single

nutritional requirement, i.e., either biotin, methionine, threonine, leucine, or thiamin, allowing the proliferation of the corresponding single mutant as well as the prototrophic type. Colonies were then picked at random and scored according to their nutritional requirements. The results are summarized in table 4. Unfortunately, it was found that the addition of methionine to the minimal medium allowed excessive growth of B^-M^-, presumably because of a degree of contamination of the methionine with biotin. This datum is, however, not essential for the argument. In general, it will be seen that the + classes are markedly and significantly more frequent than the single mutant types, with

TABLE 4

Relative frequency of various biochemical recombination classes in the cross.

$B^-M^-T^+L^+B_1^+ \times B^+M^+T^-L^-B_1^-$*

FROM PLATES SUPPLEMENTED WITH	NUMBER OF COLONIES TESTED	RECOMBINATION CLASSES FOUND				RATIO	χ^2
		TYPE	NUMBER	TYPE	NUMBER		
Biotin	70	B^-	10	B^+	60	0.17	36
Threonine	46	T^-	9	T^+	37	0.24	17
Leucine	56	L^-	5	L^+	51	0.096	38
Thiamin	87	B_1^-	79	B_1^+	8	9.88	56

* Cells of the parental types were mixed and plated into agar supplemented with the growth factor indicated. On this medium, the two recombination classes indicated on each line of the table could form colonies. Contrasting alleles only are specified; other loci, unless otherwise specified, have the "+" configuration. The χ^2 for the ratio of single biochemically deficient types to prototrophs is calculated for a comparison with the 1:1 expectation of a random segregation. As can be seen from the χ^2 values, the probability that the deviations are due solely to chance is, in each case, less than .001.

the exception of B_1^- which is nearly ten times as frequent as B_1^+. Writing the cross as $B^-M^-T^+L^+B_1^+ \times B^+M^+T^-L^-B_1^-$, these results may be interpreted as follows:

1. B^+M^+ $T^+L^+B_1^+$ more frequent than B^-M^+. Therefore B and M are linked.
2. T^+L^+ $B^+M^+B_1^+$ more frequent than either T^-L^+ or T^+L^-. Therefore T and L are linked.
3. $B_1^-B^+M^+$ T^+L^+ more frequent than $B_1^+B^+M^+$. Therefore B_1 is linked to B and M, but probably not between them.

One may therefore map these five loci onto not more than two linkage groups, according to the scheme in fig. 2a. In all that follows, the [B-M] and [T-L] combinations will be regarded as single units, since conclusive information as to their relative order has not been obtained. These data so far do not allow any conclusion to be drawn as to whether the regions B_1^-[BM] and [TL] are linked or are independent of each other, since a recombination between them is a necessary requirement for a detectable type.

TABLE 5

*Segregation of Lac and V_1 into prototrophs issuing from various parental combinations.**

PARENTS			RECOMBINATIONS			
$B^-M^-T^+L^+B_1^+$	$B^+M^+T^-L^-B_1^-$	$B^+M^+T^+L^+$	$Lac^-V_1^r$	$Lac^-V_1^s$	$Lac^+V_1^r$	$Lac^+V_1^s$
$Lac^+V_1^r$	$Lac^-V_1^s$	$\cdots B_1^+$	602 [45.8]	203 [23.1]	387 [29.4]	22 [1.7]
		$\cdots B_1^{-***}$	13 [45]	8 [28]	8 [28]	0 [0]
		$\cdots B_1^{-**}$	244 [42.8] (D)	157 [27.5] (E)	159 [27.9] (C)	10 [1.9] (triple)
$Lac^+V_1^s$	$Lac^-V_1^r$	$\cdots B_1^+$	107 [33.2]	145 [45.0]	9 [2.8]	61 [19.0]
		$\cdots B_1^{-**}$	134 [35.8] (E)	151 [40.4] (D)	9 [2.4] (triple)	80 [21.4] (C)
$Lac^-V_1^r$	$Lac^+V_1^s$	$\cdots B_1^+$	28 [23.9]	6 [5.1]	46 [39.3]	37 [31.6]
		$\cdots B_1^{-**}$	102 [25.4] (C)	7 [1.7] (triple)	201 [50.1] (D)	91 [22.7] (E)
†$Lac^+V_1^r$	$Lac^-V_1^r$	$\cdots B_1^{-**}$	128	0	33	0
†$Lac^-V_1^r$ (Y-87)	$Lac^-V_1^s$ (Y-53)	$\cdots B_1^{-**}$	134 *Lac*⁻; not scored for V_1			

* Cell mixtures of the indicated composition were plated into minimal agar plates or into plates supplemented with thiamin. B_1^+ types refer to scores of prototrophs picked at random from minimal plates.

** B_1^- refers to colonies picked at random from thiamin supplemented plates. Although predominantly B_1^- they contain B_1^+ colonies in the proportion 1:10 as may be seen from table 4.

*** B_1^-. In this series, colonies were scored as to B_1, and only the B_1^- are recorded.

The letters (C), (D), (E), refer to crossover types corresponding to the regions [*B M*]–*Lac*; *Lac*–V_1; and V_1–[*T L*] respectively, according to the map of Fig. 2d.

† Test for allelism.

On the basis of table 5, the factors V_1 and *Lac* may be brought into the argument. In addition to the joint segregations of these factors, the effect of the B_1 segregation was studied in the following way. It would be uneconomical, in view of the relative paucity of B_1^+ types, to separate these from the B_1^- by nutritional testing of colonies which appear on thiamin supplemented agar. Instead, the entire sample was regarded as B_1^- with the proviso that it might be contaminated to the extent of ten percent with B_1^+. However, it has been found that the distribution of *Lac* and *V* on colonies picked from thiamin

supplemented agar is homogeneous with the distribution in prototrophs, so that the segregation of these factors is not influenced by the B_1 segregation.

The data in table 5 show that *Lac* is inclined not to separate from *BM*, and is therefore regarded as linked to it, while there is a similar linkage of V_1 to *TL*. Since the recombination of *Lac* with *BM* is not influenced by the interchange between B_1 and *BM*, they are on opposite sides of *BM* as suggested by map 2b. Finally, a scrutiny of the interaction between the *Lac* and *V* segregations shows that these are not independent of each other, particularly because of the rarity of the least frequent class. This suggests, then, that the two linkage groups of fig. 2b be combined to give the map of fig. 2c. (The locus of V_6 on this map is obtained from additional data.) According to this interpretation, the rarity of the least frequent *Lac-V* combination stems from the fact that a triple-crossover is necessary for its production. In fig. 2d, the cross Y-40×Y-53 is interpreted according to the map, with a table citing the regions in which interchange must take place to yield the given types.

That the first seven factors to be investigated should fall in the same linkage group leads to the inference that there is only a single chromosome in *E. coli*. This inference is supported by incomplete analyses of the segregations of 8 other markers referred to in table 1. None of these factors has been found to segregate independently of the factors which have already been described as belonging to a single linkage group. The possibility that segregation interactions may, in some cases, be based upon an inter-chromosomal type of interference (compare STEINBERG and FRASER, 1944), has not been ruled out, however.

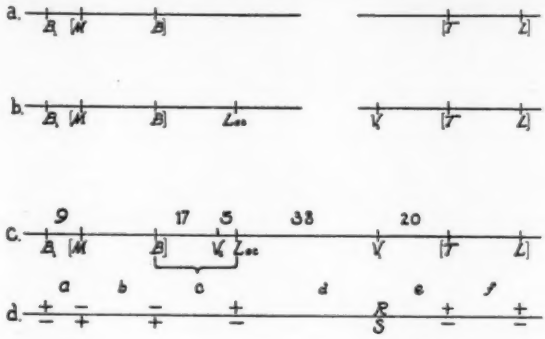
The distances recorded in fig. 2c are derived from the recombination totals in tables 5 and 6. However, the distance between [*BM*] and [*TL*] cannot be estimated directly, but only the partition of that distance among the regions *BM-Lac*, *Lac-*V_1, and V_1-*TL*. The relative frequency of the "triple-interchange" type can be used to estimate the absolute map distances, if it is assumed that there is no interference. This frequency, about 2.1 percent, is readily calculated to be consistent with a map length of between 75 and 80 units altogether either in a two-strand or a four-strand system (LEDERBERG, 1947). These values must be regarded as rough approximations, because they are extremely sensitive to error in the estimation of the proportion of the "triple" types.

Linearity

In constructing a map, and calculating distances, it has been taken for granted that there is in *E. coli* a system of linear linkage, such as has been demonstrated quite conclusively in Drosophila, and inferred in all higher organisms. What direct evidence may one bring to bear on this question?

The method which one is forced to employ in hybridizing this bacterium introduces certain complications. The classical proof of linearity is based on the additive character of distances, expressed in morgans, between loci occurring within the same linkage group. The determination of map distances is based upon a comparison between parental and new combinations of linked

genes, as determined in the progeny of zygotes selected at random. In *E. coli*, on the other hand, one is limited to the recovery of that recombination class in which there has necessarily been an interchange between certain biochemical loci, in the cases here discussed, betwen [*BM*] and [*TL*]. For this reason, it is not possible to obtain a direct measure of the absolute distance between factors which are located within this critical region, and any argument in favor of linearity which is based on the segregations of such factors may have the



Crossovers	*Recoverable type*
(*c*, *d*, or *e*)	$B_1-M+B+T+L+$
(*a*) (*c*, *d*, or *e*)	$B_1+M+B+T+L+$
(*a*) (*b*)	$B_1+M+B-T+L+$
(*a*) (*f*)	$B_1+M+B+T-L+$
(*a*) (*f*) (*c*, *d*, or *e*)	$B_1+M+B+T+L-$
(*a*) (*c*)	$B_1+M+B+T+L+ \cdots Lac^+V_1^r$
(*a*) (*d*)	$\cdots Lac^-V_1^r$
	$\cdots Lac^-V_1^s$
(*a*) (*c*) (*d*) (*e*)	$\cdots Lac^+V_1^s$

FIGURE 2.—*a*, *b*, and *c*. Mapping of genetic factors. *d*. The cross $B_1^+M^-B^-Lac^+V_1^rT^+L^+ \times B_1^-M^+B^+Lac^-V_1^sT^-L^-$ and some of the recoverable crossover classes. (See table 5.)

flavor of circular reasoning. It would be preferable to study the segregations of factors which are assigned to loci distal to the biochemical factors whose recombination is the basis of the detection of sexual offspring. The stocks with which this might be accomplished are not yet available, but it is hoped that they will be for future work.

That there does exist some sort of linkage system is made highly credible by the results of the "reverse crosses" tabulated in tables 3 and 5. The chief difficulty in proving that this system is linear has been to formulate the feasible alternatives, so that critical experiments, the results of which could discriminate between linearity and a given alternative, might be set up. Certain types of "linkage" can be disqualified by the data already at hand. For example, one might postulate that genes of bacteria are embedded in a two-dimensional

matrix, and there occasionally occurs a gene-for-gene interchange. This is equivalent to the "Konversion" theory once proposed by WINKLER (1932), to account for interchanges in Drosophila. While this type of arrangement would account for a tendency to preserve the parental configuration, it fails to explain either quantitative linkage intensities, or the interaction of segregations which is revealed by the data on *Lac* and *V* in table 5. Naturally, one could further modify the "Konversion" theory to take these exigencies into account, but in so doing one would be elaborating an exceedingly complicated theory which would, in fact, be a re-expression of a mechanical theory of linkage.

The interaction of the *Lac* and *V* segregations is perhaps the most critical datum with which a genetic system for *E. coli* can be formulated. The interaction may be expressed as follows: the frequency of interchanges between [*BM*] and *Lac* is dependent upon the interchanges between [*BM*] and *V*. Specifically, in the cross $B^+M^+T^-L^-B_1^-$ $Lac^-V_1^s \times B^-M^-T^+L^+B_1^+$ $Lac^+V_1^r$, one finds in the $B^+M^+T^+L^+B_1^+$ the following distribution of classes: $Lac^-V_1^s$ 23 percent, $Lac^+V_1^r$ 29 percent (for the parental combinations) and $Lac^-V_1^r$ 46 percent, $Lac^+V_1^s$ 2 percent (for the new combinations). With reference to $[B^+M^+]$, Lac^- is the parental, Lac^+ the interchange type. The proportion of V_1^r (representing an interchange between V_1 and [*BM*]) is different in the Lac^- and Lac^+ segregations: namely $46:23 = 2:1$ and $29:2 = 14.5:1$ respectively. This interaction between interchanges is most simply explained by the assumption that factors are located on a linear segment, so that interchanges between proximal factors also lead to the crossing over of more distal factors, barring the occurrence of additional interchanges.

Additional support for the theory of linear arrangement has been found in the segregation of V_6, summarized in table 6. It will be noted that the segregations of *Lac*, V_1, and V_6 are quite congruous in the B_1^- and B_1^+ classes. In the totals, one finds the ratios, for each factor separately, of Lac^- 78 percent; V_6^r 82 percent; V_1^s 36 percent; indicating that the first two are both linked to [*BM*] while the latter is linked to [*TL*]. V_6 cannot, however, be to the left of [*BM*] because it does not interact with B_1. If, therefore, there is a linear order of genes, V_6 must be to the right of [*BM*], and because of its greater linkage intensity, nearer [*BM*] than is *Lac*. This arrangement is indicated in the map in table 6, and in fig. 2c. The agreement of the data with the hypothesis can be examined at several points. In the first place, the single exchange types, as indicated in the table, should be the most frequent. Secondly, barring multiple exchanges, an interchange between V_6 and *Lac* should lead also to an interchange between V_6 and V_1. That is to say, the $Lac^+V_6^r$ class should be more often V_1^r than V_1^s. Finally, in view of the similarity in linkage intensities to [*BM*], *Lac* and V_6 must be closely linked. Although the "triple-interchange" types would seem to be rather frequent, reference to the table may suggest that these conditions are fulfilled. In particular, it will be noted that among the Lac^-, the ratio of $V_6^r:V_6^s$ is $94:3$, or $31:1$, while among the Lac^+, this same ratio is $10:29$, or $1:3$. This difference is interpreted to mean that *Lac* and V_6 are linked to each other, as demanded by the theory of linearity.

It is not, of course, proven that the gene order is not branched at some other

point. The most economical hypothesis at this time, however, is that there is a single unbranched chromosome as the physical basis of inheritance in *E. coli*.

Attempts to Induce Aberrations

Using a chromosomal theory as a working hypothesis, it was hoped that some verification could be found by the study of types in which the normal order of genes was disturbed. Since there is only one chromcsome (from the

TABLE 6

Segregation of Lac, V_1 and V_6.

$B^-M^-T^+L^+B_1^+Lac^+V_1^rV_6^s \times B^+M^+T^-L^-B_1^-Lac^-V_1^sV_6^r$

$B^+M^+T^+L^+$	*Lac:*	−	−	−	−	+	+	+	+	
	V_1 :	*r*	*s*	*r*	*s*	*r*	*s*	*r*	*s*	TOTAL
	V_6 :	*r*	*r*	*s*	*s*	*r*	*r*	*s*	*s*	
$\cdots B_1^+$		24	16	1	0	2	1	10	2	56
$\cdots B_1^-$**		52	42	2	0	6	1	16	1	120
Total		76	58	3	0	8	2	26	3	176
%		43	33	1.7	0	4.6	1.1	15	1.7	
Crossover region		*e*	*f*	*cde*	*cdf*	*d*	*def*	*c*	*ced*	

B_1	$\cdots M$	$\cdots B$	$\cdots V_6$	$\cdots Lac$	$\cdots V_1$	$\cdots T$	$\cdots L$
	a	*b*	*c*	*d*	*e*	*f*	
+	−	−	*s*	+	*r*	+	+
−	+	+	*r*	−	*s*	−	−

** See footnote to table 4.

genetic evidence), the only types of rearrangements would be changes leading to a series of inversion-transposition types. It was thought that such types might be detected by genetical procedures by virtue of their effect on crossing over. In particular, the occurrence of an inversion in the region $B_1 \cdots [MB]$ would be expected to have the effect of eliminating the recombination classes involving interchanges in this region. In the cross $B^-M^-T^+L^+B_1^+ \times B^+M^+T^-L^-B_1^-$ this would be equivalent to the suppression of prototroph recombinants; B_1^- types, however, would be recoverable, and allow the investigation of the extent of the changes.

Preliminary attempts to find such aberration types have, to date, been unsuccessful. The procedure was as follows:

Following treatment with nitrogen mustard (TATUM, 1946) or 20,000 r of X-rays, cells of Y-40 and of Y-53 were incubated separately for 24 hours, to allow the separation of cells or nuclei that might have been associated at the time of treatment. The cultures were then streaked out on nutrient agar

plates. Single colonies of Y-40 were picked and streaked across a nutrient agar plate. Streaks of similarly treated Y-53 colonies were made from the opposite direction, so that in the center of the plate, cells of the two types were mixed, treated colony by treated colony. The plates were incubated for 24 hours, the mixed growth scraped from the plates, suspended in sterile water and plated into minimal agar. The occurrence of colonies which would not interact to produce prototrophs, as detected by plating into minimal medium, would be an indicator that the combination was heterogeneous for an aberration. Since in these experiments, both "parents" were exposed to treatment, each plating was equivalent to the testing of two chromosomes for the occurrence of an aberration. No marked variation in the yield of prototrophs was noted in tests involving 121 mustard- and 28 x-ray-treated chromosomes. This can scarcely be regarded as an adequate sample in view of the stringent selection imposed by the technique, which might be expected to eliminate any aberration types which are even slightly less vigorous than the normal. This consideration is especially relevant in view of the "hemizygous" condition of any aberrations in the probably haploid vegetative cells. These studies will be continued.

How Many Segregants per Zygote?

In the experiments detailed in this paper, recombinants were obtained from different cell types which were exposed to each other in an agar medium. Therefore each prototroph recombinant colony seen by the experimenter marks the site of formation of a zygote. The question may immediately be raised whether there are at that site other recombination classes which, by virtue of their biochemical deficiencies, remain dormant within the prototroph colony on the minimal selective medium. This is equivalent to inquiring whether there is but a single viable product of meiosis (as in megasporogenesis in many higher plants) or more than one, as in the ascomycetes. The solution to this problem would be of special interest in relation to the possible occurrence of four-strand crossing over. In addition, if an appreciable proportion of prototroph colonies consisted of two distinct segregation types, it would be necessary to isolate these types for the collection of segregation data.

There are at least three ways in which a zygote might yield more than one haploid recombinant. Firstly, the zygote might be capable of proliferation in the diplophase (or sporophyte), leading to the concurrence of several diploid cells, each of which might undergo meiosis independently, and by chance yield several segregation types. Secondly, a single zygote might produce, after meiosis, in addition to the prototroph, the *complementary* multiple mutant class. Thirdly, in a system of four-strand crossing-over, there might be two *supplementary* prototroph recombinants differing in the segregation of factors such as *Lac* and V_1 for which the diploid was heterozygous.

Obviously, the proper investigation of these possibilities requires that one stringently avoid contamination of one colony with another. For this reason, the cell suspensions used were diluted so as to yield only about five to ten recombination colonies per plate.

Crosses were made between Y-40 and Y-53 ($B^-M^-T^+L^+B_1^+Lac^+V_1^r \times B^+M^+T^-L^-B_1^-Lac^-V_1^s$) on B_1-containing minimal agar medium. As already noted, about 90 percent of the colonies from such a cross are $B^+M^+T^+L^+B_1^-$. The theoretical complementary class would be $B^-M^-T^-L^-B_1^+$. Because of its nutritional deficiencies, it could not be expected to proliferate on the minimal medium even had it been produced after meiosis. The possibility remains, however, that a few cells of this constitution might still be present among the 10^8 or so B_1^- cells of the predominant type in a colony. By plating such colonies into medium lacking B_1 but containing biotin, methionine, threonine and leucine, the B_1^- cells would be suppressed, while the postulated multiple mutant type could form colonies and be recovered.

The experiment just described was carried out, testing 52 colonies for their content of other cell types. In general, a thiaminless colony could be shown to contain from 10–100 cells capable of forming colonies on the *B*, *M*, *T*, *L* medium. However, in each case investigated these have been shown to be indistinguishable from the Y-40 parental B^-M^- type, and must be presumed to arise from a surprisingly low degree of contamination of the colony with these cells from the heavily seeded plate. A few colonies were found which could be characterized as reversions from B_1^- to B_1^+. These experiments are then, inconclusive with respect to the occurrence of complementary genotypes in the same colony. With appropriate stocks, not as yet available, it should eventually be possible to manipulate the situation so that the complementary type could be recovered selectively, excluding both parents and the predominant recombination class.

A search for supplementary types was conducted with the same crosses, except that colonies appearing on B_1 agar were streaked out directly on EMB-lactose agar to determine whether any of them were heterogeneous for *Lac*. In some cases, a number of isolated colonies from each EMB-test plate were then also tested for homogeneity with respect to *T1*-resistance. About 90 colonies were so tested; only one colony was found containing both Lac^+ and Lac^- cells. It is impossible to be certain that, with this low frequency, the single colony which was picked was not actually derived from two distinct zygotes. These experiments cannot be considered as bearing critically on the question of the occurrence of two- or four-strand crossing over because of the absence of information concerning (a) the viability of more than one meiotic product and (b) chiasma interference. The results do, however, justify the technique of picking the prototroph colonies directly, and testing them without further purification for the collection of segregation data.

A Comparison of Sexual Recombination and Transformation

The occurrence of recombination types has been interpreted by us (LEDERBERG and TATUM 1946c, TATUM and LEDERBERG 1947) as a consequence of cell fusion, "karyogamy" and meiosis with crossing over. This is, however, not the only allowable interpretation of the general phenomenon of the occurrence of new character combinations. By analogy with the systems which have been described in pneumococci (AVERY, MACLEOD and MCCARTY 1944) and other

strains of *E. coli* (BOIVIN and VENDRELEY 1946) one might postulate that genotypically distinct cells interact not through cell fusion, but through the release of "transforming substances" diffusing through the medium. Such transforming substances would have the property of inducing or directing mutational changes in the cell receiving them so as to lead to what appear to be recombination types. Our inability to separate such postulated transforming substances from the cells themselves is not proof of their absence but could be due to their lability in our hands.

In previous publications, certain reasons were given for the rejection of the transformation hypothesis in favor of a picture of cell fusion, and so forth. It was not our intention thereby to state, with clairvoyant insight, that no investigator will be able to duplicate the results which we have reported, using instead of living cells extracts specially prepared. It is, rather, our view that since we have been able to demonstrate no appreciable point of difference between the features of gene exchange in this strain of *E. coli* and in the classical materials of Mendelian experimentation, the most economical conclusion is that the mechanisms involved are also similar. In the absence of more detailed information on the behavior of transforming systems, a critic would be free to impute to such systems all of the properties which have been found to characterize the genetic system of *E. coli*, K-12. While this would be tailoring the cloth to suit the customer, it cannot be disputed that the only conclusive method by which it could be shown that cell fusion underlies gene recombination would be a direct cytological demonstration. The rarity with which the presumed zygote occurs, however (as indicated by the low frequency of effective recombination types) is very discouraging to attempts to find and characterize the "fusion-cell," at least in the present material.

Certain genetic experiments were performed in an attempt to characterize further the behavior of this system. On the transformation hypothesis, one must attribute the rarity of the imputed transformations primarily to restricted conditions for susceptibility to the transforming factors released into the milieu. Otherwise, one would expect to find "transformations" for single factors much more frequent than those involving more than two factors. A glance at tables 4 and 6 illustrates that certain "multiple transformed" types are much more frequent than singly transformed classes. Under these conditions, one might also anticipate that genetic materials from two different kinds of cells could mix in the medium and together transform a third. In a mixture of three cell types then, one should find cases where genes from all three have combined. Using *Lac* and V_1 as markers, this type of experiment was set up in several different ways, as summarized in table 7. Pairwise, prototrophs can be formed only from biochemically distinct and nonoverlapping parents. Combinations of B^-M^- and of $T^-L^-B_1^-$ were arranged so that taken two at a time they were heterozygous either for *Lac* or for V_1 but not both. For example, a mixture of $B^-M^-Lac^-V_1^r$, $T^-L^-B_1^-Lac^-V_1^s$ and $T^-L^-B_1^-Lac^+V_1^r$ was plated. Prototrophs could be formed by recombination between either of the two latter and the former types. In one case, only V_1 would be heterozygous, and the expected types would be $Lac^-V_1^r$ and $Lac^-V_1^s$. In the other, *Lac*

would be heterozygous, and prototrophs carrying the markers $Lac^+V_1^r$ and $Lac^-V_1^r$ could be produced. The type $Lac^+V_1^s$ would not be expected unless, indeed, genetic material from all three types could combine in a sort of ménage à trois. As recorded in table 7, no instance of such a three-way combination was found in 628 tests, a different class being vacant, as anticipated, in each of the four parts of the experiment. It may be concluded that genetic factors from different cells are not freely miscible, as would be demanded by the most economical version of the interpretation of transformations.

From all the experiments so far cited, it must be concluded that if trans-

TABLE 7

Pairwise occurrence of recombination in mixtures of three components.

PARENTAL TYPES		RECOMBINANT PROTOTROPHS* $B^+M^+T^+L^+B_1^+$ or B_1^-				
$B^-M^-T^+L^+B_1^+$	$B^+M^+T^-L^-B_1^-$	$Lac^-V_1^r$	$Lac^-V_1^s$	$Lac^+V_1^r$	$Lac^+V_1^s$	TOTAL
$Lac^-V_1^r$	$Lac^+V_1^r$ $Lac^-V_1^s$	173	49	4	0	226
$Lac^+V_1^r$	$Lac^-V_1^r$ $Lac^+V_1^s$	16	0	7	28	51
$Lac^+V_1^s$	$Lac^+V_1^r$ $Lac^-V_1^s$	0	136	37	40	213
$Lac^+V_1^s$ $Lac^-V_1^r$	$Lac^-V_1^s$	65	48	0	25	138
					Total	628

* Mixtures of the three types indicated in each experiment were plated into thiamin-containing agar. The prototrophs are therefore a mixture of B_1^- and B_1^+ types, as indicated in table 4, footnote.

forming factors are operating in this system, the diverse factors (or genes) are not independent of one another, but are grouped in separate and immiscible parcels. Such parcels would also be potentially capable of transmitting all of the genetic factors of a cell, so that there seems to be no compelling reason why such a parcel, speaking purely genetically, could not be regarded as a gamete. MULLER (1947) has interpreted the pneumococcus transformation in terms of "still viable bacterial chromosomes or parts of chromosomes floating free in the medium . . . these have penetrated the capsuleless bacteria and in part at least, taken root there, perhaps after having undergone a kind of crossing over with the chromosomes of the host." It remains to be seen whether this interpretation will be upheld by further studies on factor interaction in *bona fide* transforming systems.

Several attempts were made to determine whether "transforming activity" could be separated from the living cell under conditions comparable to the

platings in minimal agar medium, or after extraction of cells by BOIVIN'S method (BOIVIN *et. al.*, 1946). No activity was found in the supernatant of a suspension of Y-40 and Y-53 together or separately in the same minimal liquid medium to which agar is added for plating experiments. The only manipulation involved here consists of the removal of most of the bacteria by ordinary centrifugation. It could thus be shown that the "activity" was associated with the cells. Equally negative results characterized attempts to reveal transforming activity on culture filtrates and cell autolysates prepared as crude fractions according to BOIVIN'S procedure. Finally, the addition of desoxyribonuclease in a final concentration of .05 mg/ml to the mixing and plating medium had no effect on the number of prototrophs which appeared in the cross of Y-40 and Y-53. Tests for the destruction of enzymatic activity under these conditions were, however, not done.

The conclusions which we draw from these experiments are (a) that the existence of transforming factors is exceedingly unlikely and (b) it would be not worthwhile to go to extreme trouble to attempt to isolate such factors from this system until the study of *bona fide* transforming systems has progressed sufficiently that the genetical criteria already discussed might be applied.

DISCUSSION

Regardless of the stand that one takes on the issue of invisible zygotes versus non-extractable transforming factors, it can be asserted that *E. coli* K-12 provides a useful tool for genetic analysis. The use of biochemical mutants as parents allows crosses which are nearly as well controlled as in Neurospora. The segregational behavior of mutant factors seems to be closely analogous to that of higher forms, and seems to compel their admission into the same arena as the genes of Drosophila. However, it would be premature to transfer these conclusions to other genetic characters of other microorganisms, each of which must be examined on its own merits.

It may be wondered that the apparent recombination rate is so low. However, this is possibly not to be attributed to any sexual imperfections of *E. coli*, but to the method of enumeration. It seems likely that an analogous comparison of the number of somatic and generative cells in an organism like the oak-tree, or man (especially the female of the species) would give ratios similar to those prevailing in *E. coli*. It is also possible that the optimal conditions for zygote formation or germination have not yet been achieved and that by special procedures the rate of zygote-formation may be accelerated to the level where there might be some hope of finding it in the field of the microscope.

Attempts to detect recombination in two other strains of *E. coli*, B (DEMEREC and FANO, 1945) and L-15 (ROEPKE, LIBBY, and SMALL, 1944) by analogous methods have been unsuccessful (LURIA, 1947, TATUM and LEDERBERG, 1947). At least two strains then must be classified with the "*Fungi Imperfecti*." This dismal conclusion is, however, illuminated by the fact that many heterothallic species have been eliminated from the *Fungi Imperfecti* with the discovery of the appropriate opposite mating-types. At the present time, one scarcely knows where to begin to look for the bacterial analogy. The

application of genetic techniques to the elucidation of unusual life-cycles in diverse bacteria (BRAUN and ELROD, 1946, DIENES, 1946) cannot fail, however, to be most fruitful.

The evolutionary significance of gene recombination has been made so widely familiar by DOBZHANSKY's book (1941), and adequately discussed, more recently, by MULLER (1947), that it would be impertinent to do more than simply refer to these papers.

SUMMARY

The recombination of genetic factors and their segregation into prototroph recombinants of *Escherichia coli* have been studied. It was found that genetic markers behaved as if they were part of a system of linked genes. Some evidence for linear order of genes was obtained. Each of 15 factors studied fell into the same linkage group. Data are given in detail for the segregation of factors involved in the biosyntheses of biotin, methionine, threonine, leucine, or thiamin; in the fermentation of lactose, and in resistance to bacterial viruses *T1* and *T6*. On the basis of these data a tentative 8-point genetic map of the chromosome of *E. coli* is presented.

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GENETIC ANALYSIS OF AN ANOMALOUS SEX RATIO CONDITION IN DROSOPHILA AFFINIS

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INTRODUCTION

GENETIC alteration of the normal sex ratio in Drosophila by a disturbance of the meiotic processes provides a means for the investigation of chromosome mechanics, somewhat analogous to the inferential determination of the properties of the wild-type gene by a study of its mutant alleles, and further presents problems of evolutionary importance in those cases where the genetic system responsible is found in natural populations and therefore cannot be dismissed as a laboratory curiosity. Perhaps the most striking example satisfying both these criteria is that of the "sex ratio" gene, which causes males bearing it to produce few or no male offspring. This "sex ratio" gene has been found in a member of the *affinis* group (STURTEVANT in MORGAN, BRIDGES, and STURTEVANT, 1925), in *obscura* (GERSHENSON, 1928), in *athabasca*, *azteca*, *affinis*, and *pseudoobscura* (STURTEVANT and DOBZHANSKY, 1936) and in *melanica* (STURTEVANT in STURTEVANT and NOVITSKI, 1941; SPENCER, unpublished).

GERSHENSON has shown that meiosis of *obscura* females heterozygous or homozygous for this X chromosome gene proceeds normally but that males hemizygous for it produce nearly all X sperm instead of the normal 50 percent. STURTEVANT and DOBZHANSKY have localized the factor (or factors) responsible on the right limb of the X chromosome in *pseudoobscura;* a cytological investigation revealed that the X chromosome of "sex ratio" males undergoes an equational division at each meiotic division, the Y chromosome usually degenerating so that each spermatid generally receives an X chromosome, but no Y.

The anomalous sex ratio to be described here is one which results in the production, by certain *affinis* males, of only male offspring. This phenomenon has been named "male sex ratio" (abbreviated hereafter as MSR), to distinguish it from the opposite condition described above.

MATERIALS

The mutant genes in *affinis* used here include: white (*w*) and miniature (*m*), on the standard sequence of the X chromosome; ascute (*asc*), scarlet (*st*) and hairy (*h*) on the inverted sequence of the X chromosome; jaunty (*jt*) and net (*nt*) on chromosome B; pinklike (*p*) and cinnabar (*cn*) on chromosome C;

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rugose (*rug*) on chromosome E. More detailed information about the nomenclature of the chromosomes may be found in STURTEVANT and NOVITSKI (1941) and NOVITSKI (1946). The Y-autosome translocations made use of in the latter part of the work were selected from the translocations which yielded the original correlation of salivary gland chromosomes and linkage groups in this species.

"SEX RATIO" IN AFFINIS

Since, as will be shown later, MSR is associated genetically with "sex ratio," some of the genetic characteristics of the latter must be considered first. STURTEVANT (1940) has recorded for *affinis* two widely distributed sequences of the X chromosome, "standard" and "inverted." The "sex ratio" effect that has been investigated here is produced by an X chromosome carrying a third sequence. This sequence reduces the crossing over between hairy (*h*) and scarlet (*st*), 62 units apart on the "inverted" sequence, and between white (*w*) and miniature (*m*), 35 units apart on the "standard" sequence, to practically zero. An examination of the salivary gland chromosomes of heterozygotes for the "sex ratio" X chromosome and "standard" show that the former sequence cannot be a simple crossover derivative of the other two.

The ratio of the sexes obtained from "sex ratio" males is quite variable, although the source of all the "sex ratio" X chromosomes used here can be traced to one such X chromosome found in a series of cultures manifesting the MSR effect. In one set of 8 cultures, an average of 170 ♀♀ to .6 ♂♂ per culture was obtained; in another set of 18, the average ratio per culture was 97 ♀♀ to 46 ♂♂. These data suggest the presence of one or more modifying factors on the other chromosomes, but an analysis of these has not been attempted. The possibility of mistaking "sex ratio" cultures for normal ones and vice versa has been reduced by using whenever possible only "standard" and "inverted" sequences marked with recessive mutants, their respective wild-type alleles automatically marking the "sex ratio" sequence.

DISCOVERY OF THE MSR PHENOMENON

During the course of the translocation studies above mentioned three cultures, 1-17, 1-21 and 1-24, yielded only male or almost only male offspring. The nature of the matings which showed this effect may be described as *cn rug* ♀♀ × F_1 (*cn rug* ♀♀ × Woods Hole X-rayed ♂♂) ♂♂. Two transfer cultures of 1-17 produced a total of 12 ♀♀ and 208 ♂♂, culture 1-21 produced 10 ♂♂ and culture 1-54 produced 25 ♂♂. This phenomenon was later observed by PROFESSOR A. H. STURTEVANT in the F_2 from a pair mating of F_1 individuals from a cross of a *p* ♀ by a *cn* ♂. Only 1 ♀ but 64 ♂♂ constituted the entire progeny of this bottle.

The original discovery of MSR in crosses involving irradiated chromosomes had suggested that the causal factor was an induced mutation or a chromosomal aberration. The later appearance of this condition in cultures derived only from wild strains negates that possibility. The fact that all four crosses showing this effect involve stocks carrying *cn* is strongly suggestive of the presence in the *cn* stock of the genes responsible.

THE GENETIC COMPOSITION OF MSR MALES

The preliminary crosses designed to isolate the aberrant genetic system responsible for MSR were rather ineffective. Several pertinent points were, however, established. The appearance of MSR in cultures in which the female parents were of the compositions *w*, *h st*, *asc st*, *h st/w*, *h st/+*, *w/+*, and *jt net* showed that the genetic composition of the male alone was the determining factor. Inbreeding experiments indicated that the genetic basis of MSR was not simple, and that "sex ratio" was in some way associated with it. The rather high sterility of MSR males and the unisexual inclination of the cultures from both MSR and "sex ratio" males made inbreeding difficult. Outcrossing individuals from the strains known to contain, to some degree, the MSR complex to mutant strains for the purpose of marking the chromosomes generally led to the loss of the MSR effect, as tested by subsequent inbreeding. In only one case was the outcrossing followed by a recovery of the MSR effect; the strain extracted has been the basis of all later work.

The single female appearing in the F_2 of the mating of a *p* ♀ × a *cn* ♂ was crossed with a *w* male. Tests by mating to *w* females of 16 of the male offspring from this cross showed clear evidences of the presence of a "sex ratio" X chromosome (to be denoted as *sr* hereafter) in five of the males. Females of the constitution *sr/w* from the culture showing the most extreme sex ratio (101 ♀♀ : 1 ♂) were mated to *w m* males in order to obtain a stock carrying the *sr* X chromosome in a balanced condition. After one generation of inbreeding (*sr/w m* ♀♀ × *w* ♂♂) males carrying *sr* were tested and were found, in a few cases, to produce only male offspring; the majority of the fertile cultures showed the typical "sex ratio" phenomenon. A large series of matings of the type *w/sr* ♀♀ × *w m* ♂♂ was set up, with a subsequent selection of those in which at least some of the males carrying the *sr* X chromosome produced only male offspring. The strain finally derived, of which all fertile males having the *sr* X chromosome also showed the MSR effect, regardless of the genotype of its mate, was maintained by selection of *sr/w m* ♀♀ and *w m* ♂♂ as the parents for each generation.

An obvious conclusion is that the *sr* X chromosome is a necessary prerequisite for the MSR effect of a male. Never has a male with "standard" sequence (marked by *w*) produced only male offspring; certain cultures involving *h st*, in which all the *sr* males from a *h st/sr* female were MSR and all *h st* males were not, indicate that the "inverted" sequence is also not involved in MSR.

Several types of matings indicate the nature of the difference between "sex ratio" males and MSR males. If a female of the constitution *w m/sr* is outcrossed to certain unrelated strains, as *asc st* or *jt net*, the F_1 *sr* males invariably show the typical "sex ratio" effect. MSR males can be found among the *sr* males of the F_2. When the male parent comes from the same inbred strain as the female parent, all the *sr* males show MSR.

The above results can be explained by the assumption that an autosomal recessive gene (*a*) present in the homozygous state in *sr* males (*sr*, *a/a*) converts it from a "sex ratio" male into a MSR male. Assuming that this factor

exists, there is no evidence that it has any effect in the heterozygous state.

In order to demonstrate the existence of the *a* gene, an attempt was made to localize it on a particular chromosome. Pair matings of *w m/sr* females, of the inbred MSR strain and so with the hypothetical constitution *w m/sr, a/a*, were mated to males carrying *jt net* (chromosome B), to males carrying *cn* (chromosome C) and to males carrying *rug* (chromosome E). Stocks of the constitution *w jt net*, *w cn* and *w rug* were derived from this mating to eliminate the unmarked X chromosomes from the autosomal mutant stocks. The factor *a* might be present in these stocks (1) if selection for the mutant genes did not eliminate it by virtue of their location on different chromosomes or by virtue of an appreciable amount of crossing over between it and the mutant gene if they were on the same chromosome, and (2) if the *a* factor was present in the original autosomal mutant individuals mated to *w m/sr, a/a* females. This latter possibility was most serious for the cross involving *cn*, because of the evidence from earlier crosses which had indicated the presence of the *a* factor in the *cn* stock. Tests of F_1 *sr* males, which should reveal whether the particular mutant individuals used carried the *a* factor, either in the homozygous or heterozygous state by the appearance of the MSR effect either in all or in half the males, gave negative results.

Males from the multiple mutant stocks were then mated to *w m/sr, a/a* females and F_1 *sr* males were tested for the presence of the homozygous *a* factor in the way described above as a test for the presence of the *a* gene in the autosomal mutant stocks.

The results of these tests were quite striking: the *w rug* strain was homozygous for the *a* gene, the *w cn* strain was heterogeneous for it, and the *w jt net* strain did not carry it at all. A strain of the composition *w m/sr, a, rug* was immediately obtained; a comparable strain homozygous for *cn* instead of *rug* was obtained within two generations. The attempt to obtain the comparable strain carrying *jt net* failed completely; this suggests that *jt net*, and *a* were on homologous chromosomes and that selection for *jt net* automatically eliminated *a*.

Corroboratory evidence that *a* is located on chromosome B has been obtained in another way. Segregation of a Y-autosome translocation in a male heterozygous for it is such that the chromosome which has exchanged fragments with the Y chromosome will generally be present in all males. Consequently, repeated backcrossing of such males to females carrying marker genes on the chromosome corresponding to that involved in the translocation is ineffective; the males will always be heterozygous for those chromosomes involved.

With the series of Y-autosome translocations available in *affinis*, such a test is relatively simple. Males carrying the translocations involving chromosome B (TY-4A), involving chromosomes B and C simultaneously (TY-3B), involving B and E (TY-4E), and involving chromosome E (TY-2A, TY-2B) were mated individually to females of the composition *w m/sr, a*. F_1 *w m* males, heterozygous for the translocation and for *a*, were backcrossed to *w m/sr, a* females. If the gene *a* is borne by the chromosome corresponding to the one in-

volved in the translocation, all the *sr* males from this cross must be heterozygous for *a* and so will behave as "sex ratio" males. If the gene *a* segregates independently of the translocated chromosomes, half the *sr* males of the backcross generation will be heterozygous for *a* and behave as "sex ratio" males, the other half will be homozygous for *a* and consequently may be expected either to be MSR or to show a characteristic sterility. Another possibility is that the translocated chromosomes of any given aberration may themselves carry *a* rather than its wild type allele, since MSR was first discovered in that set of cultures from which the translocations were derived. In this case such backcrossing should lead to *sr* males of which all would be expected to show the MSR effect instead of "sex ratio." These two expectations, males all of one kind or of two kinds have been represented in table 1 by the numbers 1 and 2, respectively, for the translocaions used, when *a* is assumed to be located on each of the three long autosomes.

TABLE 1

The numbers of the kinds of males expected from back crosses to w m/sr, a females of males carrying certain translocations, considering individually the various possibilities for the location of "a" on the three long autosomal chromosomes.

TRANSLOCATION	POSITION OF *a*		
	IF ON CHROMOSOME B	IF ON CHROMOSOME C	IF ON CHROMOSOME E
Y-2A	2	2	1
Y-2B	2	2	1
Y-3B	1	1	2
Y-4A	1	2	2
Y-4E	1	2	1

From these crosses, males of two different kinds of behavior were actually produced. In the order of the translocations as given in table 1, the different types of male per series were 2, 2, 1, 1, 1 or exactly the distribution expected if *a* is located on element B as previous observations indicated.

The two distinct types of males were not "sex ratio" and MSR, however, but "sex ratio" and normal. All of the cultures (17) of the TY-3A series were clearly "sex ratio," the average number of females and males per culture being 77 and 4.5 respectively. The 50 cultures of the TY-4E series showed an average of 70 females and 4 males. The 49 cultures of TY-4A, on the other hand, gave an average of 95 females and 85 males. In the TY-2A series nine cultures were "sex ratio"; four were normal; three were doubtful and three were sterile. In the TY-2B series, 22 were "sex ratio"; 18, normal and 13 sterile.

The conclusion to be drawn from the above results is that the presence of a Y translocation in a male of the composition *sr a* inhibits the male-producing mechanism so that normal offspring are produced. It follows also that the Y-4A translocation must have the *a* gene on the translocated chromosome B.

Because of this unexpected effect of the Y chromosome, the possibility that

a certain type of Y is involved in the MSR complex must also be considered. With the data available, this cannot be excluded conclusively. However, outcrosses of the inbred MSR strain were generally made using females, which should ordinarily not carry the Y of the MSR strain. The MSR males of the F_2 have the Y of the parental males which, in these crosses, come from a large variety of strains not showing MSR. The specific Y might, of course, be introduced by the parental female if she were XXY. However, since the two X's of such a female have inversion differences (standard/*sr*), one might reasonably anticipate a small percentage of secondary non-disjunction which was in fact not observed although the crosses for the most part involved markers which would have revealed it.

THE CHARACTERISTICS OF MSR MALES AND THEIR PROGENY

During the course of this work, a total of 95 fertile cultures involving MSR males has yielded an average of 25.5 individuals per culture. Females appeared in ten of the cultures with an average of 3.2 per culture that produced females. This gives a total sex ratio of 76.8 males per female.

The male offspring are morphologically normal. They carry an X chromosome of the mother (*w/h st* ♀♀ × MSR ♂♂→*w* ♂♂ and *h st* ♂♂). A cytological analysis of spermatogonial metaphases of three progeny of MSR males crossed to females from the same inbred line revealed that the F_1 males either may (one case) or may not (two cases) receive the Y chromosome of the father. This observation is in agreement with the fact that both *w* and *h st* males produced from such crosses tend to be sterile more often than fertile (in one series, for instance, 22 fertile males carrying "standard" or "inverted" sequences, to 38 sterile ones). The ventral receptacles of the females from four such sterile cultures were examined and proved to be devoid of sperm.

The occasional female offspring of a MSR male have one X chromosome from each parent (*w* ♀ × MSR ♂→mostly *w* ♂♂ rare *w/sr* ♀♀). Whether or not they also may have the Y of the male parent has not been established. Such females are normal, with regard both to appearance and to fertility.

When females mated to MSR males are dissected and the ventral receptacles examined, copious quantities of sperm are generally (seven cases out of eight) found therein, although such females have usually produced no or very few offspring. The sperm, if capable of fertilization, must have a lethal effect upon the zygote. The recessive nature of the factor *a* indicates that this potential lethal effect originates during spermatogenesis rather than at the time of fertilization. This time of origin is also suggested by the fact that the MSR complex has as its basis the *sr* X chromosome, which exerts its influence during spermatogenesis also.

GEOGRAPHICAL DISTRIBUTION OF THE *sr* X CHROMOSOME AND OF *a*

The *sr* X chromosome has been found in strains from Woods Hole, Massachusetts; Coffeyville, Kansas; Gatlinburg, Tennessee (STURTEVANT, 1940); it has also been found in the stock of *p* (origin, Austin, Texas). The factor *a* can be traced in various crosses to the stocks of *p*, *cn*, *h st* (origin ambiguous, in-

cluding strains from both Gatlinburg, Tennessee and Woods Hole, Massachusetts), and also in wild type strains from Plano, Texas and from Woods Hole, Massachusetts.

From these points of distribution it may be surmised that both the *sr* X chromosome and the *a* factor are present to some extent over the entire range of *affinis*. However, additional data on the geographical distribution of these factors do not confirm this surmise. The data were obtained when the loss during the war of all stocks homozygous for either or both of these elements made necessary an attempt to resynthesize the MSR complex. For this purpose there were analyzed the Plano and Woods Hole strains and twenty other wild type strains recently collected in the Southeast. The *sr* X chromosome used was derived from a Woods Hole stock. By the use of an X-ray induced dominant *Minute* mutation on chromosome B, it was possible, in three generations, to make individual chromosomes B homozygous in the presence of the *sr* X chromosome. Because of sterility only 29 chromosomes out of a total of over 130 started were completely tested. These included: one chromosome B from each of seven strains from Alabama, six strains from Georgia, and five strains from Florida; four samples from the Woods Hole stock and five from the Plano stock. In none of these tests was there any evidence of the MSR effect, although typical "sex ratio" appeared in the strains from Georgia and Mississippi. The absence of the *a* factor from the older Plano and Woods Hole stocks was not surprising, for it was anticipated that the lowered fertility characteristic of the homozygotes would lead to the eventual elimination of *a* from the heterogeneous laboratory stocks. The absence in the newer strains, however, must mean that either the gene is not as frequent in wild populations as previous observations indicate, or that its distribution does not include the southeastern area.

DISCUSSION

The behavior of populations of those species of Drosophila having factors altering the sex ratio is difficult to understand. From the evidence obtained from laboratory cultures, the *sr* X chromosome should automatically increase in frequency each generation and it can be inferred that wild populations in which this type of chromosome is found should soon become almost completely homozygous for it (GERSHENSON, 1928). Such a population, the few males in which would produce all or almost all female offspring, would remain in constant danger of extinction. STURTEVANT (unpublished) has suggested that the division of Drosophila species into subpopulations may be of significance in this respect. If the *sr* X chromosome is present in some of these subpopulations, it may frequently lead to their complete elimination. Consequently the frequency of the *sr* X chromosome in the total population may remain at a low level. In this connection it may be pointed out that while the 1:1 ratio of sexes characteristic of so many bisexual species is, fundamentally, a condition imposed by the meiotic mechanism and fulfills the basic requirements for survival it does not necessarily represent the optimum ratio, which may be quite different from one species to another, as well as within a species in different

ecologies. The low male:female ratio expected of subpopulations carrying *sr* may impart up to a point a selective advantage.

Since half the autosomal genes in a population come from each sex, the genetic contribution per individual to the succeeding generation is greater for the less prevalent sex. If, as is ordinarily true, the gene frequencies are the same for both sexes, this consideration has little effect on the composition of the following generation other than the greater statistical variation of gene frequencies in smaller populations. However, any genotype which tends to be more frequent in one sex than the other when the sex ratio is unequal undergoes a change in frequency from one generation to the next even though there is no obvious selective advantage to that genotype. For instance, a new allele present in a single male which mates with nine females constitutes only five percent of the alleles at that locus in that population of ten individuals but would ordinarily reach a frequency of .25 in the following generation. This 500 percent increase is obviously not caused by the greater selective advantage of the allele but by the greater reproductive efficiency of the male by virtue of the inequitable numbers of the two sexes.

Using the above argument, we may consider the situation in a population carrying the *sr* X chromosome. If in that population any new genotypes were to arise by mutation or recombination which allowed males carrying the *sr* X chromosome to produce some male offspring, the additional male offspring would carry the genotype not with its original frequency, but with one considerable higher. The frequency in the total of males would therefore increase, and their greater reproductive efficiency would lead to a considerable increase in the frequency of that genotype in the following generation. The increase would, fortunately for the population, be greater the greater the deficiency of males.

The customary criterion for the identification of the *sr* X chromosome in a male is the absence of male offspring. This unfortunately excludes identification of suppressors, unless they act rather weakly. Nevertheless, evidence that suppressors, probably recessives, actually exist has been found in *affinis* (above) and *pseudoobscura* (STURTEVANT and DOBZHANSKY, 1936). The deleterious effects of a very low male:female ratio might be circumvented by the conversion of "sex ratio" into "male sex ratio" but the efficacy of the factor *a* investigated here in accomplishing this in nature is open to question since the laboratory tests indicate that just those males which would serve as the agents of a frequency increase are largely sterile. However it is noteworthy that, besides the male-producing gene found in *D. melanogaster* by GOWEN and NELSON (1942), which may or may not have evolutionary significance, a rather typical "male sex ratio" effect has been found in *D. pseudoobscura* independently by K. J. MAMPELL (unpublished) and B. WALLACE (unpublished), in the latter case involving the *sr* X chromosome.

SUMMARY

The production of only male offspring by certain males of *Drosophila affinis* has as its basis a genetic constitution involving the "sex ratio" X chromosome,

which ordinarily causes males carrying it to produce only daughters, and a recessive gene on chromosome B. In the presence of Y-autosome translocations the system yields a normal sex ratio. It is suggested that the factors yielding such aberrant sex ratios have an evolutionary significance in allowing certain populations to approach an optimum sex ratio with the possibility of an immediate check should the optimum be exceeded and the survival of the population endangered.

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